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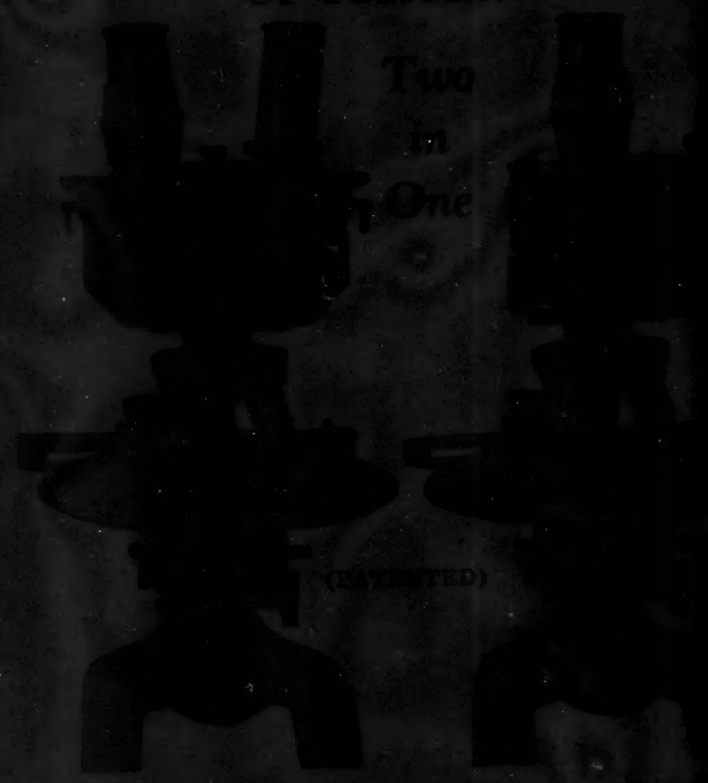

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AN ACTINOMYCETE THE CAUSE OF SOIL ROT OR POX IN SWEET POTATOES

J. F. ADAMS

During the past six years investigations have been conducted on the cause of pox or soil rot of sweet potatoes. The results of these investigations are such as to justify a preliminary account pending more complete report on the problem, study of which is being continued.

Studies of the pox disease of sweet potatoes were undertaken in 1920 in order to confirm the results of Elliott (1), who first reported that the disease was caused by a slime mold, *Cystospora batata* Ell. Elliott's results were later confirmed by Taubenhaus (13). The author's initial studies in duplicating the work of Elliott and Taubenhaus gave negative results in recovering or locating a slime mold from typical pox lesions on sweet potatoes. Mature pox lesions for cytological study were differentiated with Flemming's triple stain. Studies of these mature lesions on fleshy roots failed to reveal the presence of any foreign organism resembling a slime mold. It was reported (4) that "Products of metabolism and the results of cellular disturbances of unknown cause were consistently observed and in part assumed the morphological character of the so-called cysts, as described by Elliott. These cyst-like bodies possessed no differentiated contents, and were interpreted as probably the products of metabolism in the form of reserve substances." Further work by the writer (5), reported in 1922, failed to confirm the results reported by Elliott (1) and Taubenhaus (13) that pox of sweet potatoes was caused by *Cystospora batata* Ell.

Manns (6) reports that "In 1914 we suspected the cause of pox may be due to an Actinomycete found associated with the disease in our cultures, but results were not consistent." Taubenhaus (13), then an assistant in the department, reported upon these findings in 1918. Manns (6) found by unmounting pox material which had been stained by Elliott with Flemming's triple stain, and restaining with Ziehl's carbol-fuchsin, that in every case there was evident an Actinomycete, which may have been the cause of the disease. An experiment was established by growing sprouts in Mason jars to learn the influence of lime and manure on increasing the disease. Ground lesions of mature pox and scrapings from culture plates were used

by Manns for inoculation of sprouts in two jars, and he reports in the case of ground-lesion inoculation that "small roots were eaten off with small black lesions and one large root was rotted off." In the case of scrapings from culture plates for inoculation, it is reported that "several pox pits showed on the main roots and many rootlets were injured." This experiment was run for over six months and no duplication of work on reisolation is reported. No controls were used with soil and manure to which were added the equivalent amount of straight agar. The introduction of agar alone into such a mixture of soil and manure would naturally force into growth any fungi and bacterial flora present and produce conditions inhibitive to normal root development.

In writing of the work conducted during 1923, it was stated (6) that bacteriological staining methods were employed and found to be superior to Flemming's triple stain for differentiating organisms in the necrotic tissue of pox lesions. The safranin in the triple stain possesses only slight affinity for a fungus which to all appearances resembles an Actinomycete in its morphological characters. Ziehl's carbol-fuchsin stain was found very efficient, but for careful differentiating it was necessary to destain. A stain for bacterial flagella (11) was found very efficient. A fungus that is easily stained by the methods described above was found consistently in the lesions on fleshy roots. The necrotic tissue was found invaded with an abundance of the mycelium. The mycelium was not widely distributed but appeared in the necrotic tissue in definite pockets.

In 1925, Manns (7) reports as follows: "By improved cultural technique we have been able to take this Actinomycete from pox lesions in pure culture by growing the crushed lesion in straight agar medium, 1.2 per cent, in the absence of all nutrients. Such a medium inhibits most organisms, and *Actinomyces poolensis* pushes out almost pure. We have been able to prove its activity in producing 'soil sickness' by placing the contents of a dozen culture dishes in a quart mason fruit jar using sterile sand and proper sweet potato fertilizer." The two inoculated sprouts used for illustration show a complete collapse of the zone of root growth as well as retarded growth but no pox lesions such as are found under natural conditions of root infection in the field.

Further cytological studies (8) were continued with the pox lesions during 1924. Material showing lesions only on the rootlets was used, the object being further to confirm previous work and the results of studies with mature lesions on fleshy roots. Consistent evidence was found of the presence in lesions on rootlets of apparently the same pathogene as was found in the lesions on fleshy roots. The characters of this fungus from a cultural and morphological study are identical with those of the genus

Actinomyces. In addition to the stains previously used, it was found that Bismarck brown is very satisfactory for differentiating the pathogene. The fungus was always found associated with tissue, principally in the cortex of young roots that showed complete collapse. There was little evidence of any progressive development of the pathogene, as the lesions were very restricted on the rootlets, as in the case of infection on fleshy roots.

Some direct inoculation experiments were carried out with two strains of *Actinomyces* sp. isolated by Dr. Manns. Tall pyrex beakers of 1-liter capacity were used for this work with a layer of moist absorbent cotton for a medium, and the beakers were plugged with cotton. Sprouts grown from disinfected sweet potatoes of the Big Leaf Up River and Vineless Jersey varieties were used. Clean sprouts were secured, thoroughly washed in distilled water, and placed in the sterile beakers. There was no difficulty in maintaining the sprouts for a period of four weeks and more. Four series of four beakers each were protected from the light by being wrapped in black paper. The inoculum used in this experiment was from colonies on agar plates and was placed directly on the primary and secondary roots. Room temperature, approximately 68° F., and a temperature of about 85° F. were used. In only one instance did a lesion develop that resembled a pox lesion. The result of this experiment was considered negative.

In 1926 further work was reported (9) upon as follows: Eight roots showing abundant lesions were selected, carefully washed in distilled water, placed in sterile beakers, and submerged in distilled water. The water was changed twice a week. For a period of about four weeks thereafter the roots showed no evidence of breakdown. The experiment was started October 24, 1925, and this series of roots removed for culture isolations November 20. A series of four lesions was removed from each root and cultured on plain agar, which had proved most satisfactory in previous studies for the pathogene under manipulation. With this series of 40 plate cultures, 8 additional plate cultures were made with lesions from untreated roots as a control. It was found at the end of eight days that the plates from lesions of the potatoes which had been submerged showed on the average 50–75 per cent more colonies of the Actinomycete than the control plates. This proved that the incubation of the pox roots in water was a factor in activating the fungus. However, in no instance was there evidence that the organism had progressed so as to extend the original limits of development of the lesions. This particular character may be associated with the resistance of the root or the original protective zone of tissue cutting out the lesion. According to our observations, inception of the disease under field conditions occurs with young, actively growing secondary or fleshy roots and tissue, or at the eye points or rootlets from primary fleshy roots. This experiment was then followed with some direct inoculations.

Eight disease-free fleshy roots were disinfected, carefully washed, and placed in sterile beakers with moist cotton for forcing of roots, on October 24, 1925. Roots were well established in four weeks. Lesions from poxed roots that had been incubated in water cultures as previously described were then placed at the point of origin of the rootlets. In 10 days diseased areas were observed at points of contact of the inoculum which looked like typically mature pox lesions. The resulting diseased areas or lesions with one exception were light to dark brown in color and not more than $\frac{1}{8}$ in. in diameter and non-progressive. With one exception in the 18 inoculations, the resulting lesions were typical in every respect of small, developing pox lesions. The lesions resulting from these inoculations as well as the inoculum were plated out on plain agar on December 1. In seven days the typical Actinomycete under observation appeared in all plates. A series of plates was also made from dried pox lesions for control. The colonies were more abundant in the plates made from the newly developed lesions and from the inoculum than in the plates made from dried material. This further confirms the evidence previously reported that incubation of the lesions activated the pathogene.

In another experiment a disease-free root was disinfected and planted in sterile soil. This was kept moist with distilled water and forced until emerging root points were first showing. The soil was removed, and on the upper surface at nine different emerging root points, direct inoculations were made with pox lesions that had been soaked for 13 days in distilled water. Inoculations were made December 20, the inoculation court marked with a circle of India ink, and then the soil was carefully replaced so as not to disturb the pieces of inoculum. The resulting lesions were examined on January 11, 1926, and removed for plating cultures. In all except one instance the lesions were restricted, pitlike, and non-progressive. The results were positive with respect to production of diseased lesions. The one exception in this inoculation experiment was a lesion in which the soft-rot fungus was associated with the Actinomycete so that the resulting lesion was progressive as compared with the remaining eight which appeared as young pox lesions. The removed lesions were divided into three pieces for crushing and plating out in plain agar and nutrient glucose agar. The same specific Actinomycete under observation appeared in the plate cultures, but for some unexplained reason the colonies were not so abundant as those resulting from previous inoculations. It is possible that the pathogene had not sporulated or developed to the same extent as in the previous inoculation work.

The results of these direct inoculations indicated the presence of an organism that was pathogenic and capable of producing lesions typical of the pox disease. During the fall of 1926, fresh specimens of the pox disease

on fleshy roots of sweet potatoes were collected for more detailed isolation studies. Plain agar was used for the isolation work, as this medium eliminates much of the contamination including bacteria and other fungi naturally following the initial collapse of the host tissue. The diseased fleshy roots were thoroughly washed in distilled water and then kept in a moist chamber for nine days at a temperature of 33° C. Typical pox lesions were removed and crushed under sterile conditions and plated out in plain agar. The colonies of the *Actinomyces* sp. showing growth on these agar plates were much more numerous than colonies from finely crushed lesions that were not kept in the moist chamber. This procedure again appears to have activated the *Actinomyces* in the lesions. The isolation work failed to show a consistent appearance of any single type of *Actinomyces*. It was necessary to incubate the culture plates at a temperature of 33° C. for maximum growth of the colonies. The colonies or growth from diseased tissue was evident in the poured plates within five to eight days. Three types of *Actinomyces* were isolated and carried as pure cultures for further inoculation work. These three types varied perceptibly in growth and color characters on Ashby's, cornmeal, synthetic, Endo, and nutrient agar. Inoculations were first started on fresh slices of fleshy sweet potato roots in sterile agar plates. One of the three types was found to be capable of producing a restricted lesion, indicating that it was pathogenic. This particular type was named for convenience, *Actinomyces p.*, and will be referred to as such in the discussion as the *Actinomyces* causing pox of sweet potatoes.

A cultural difference was established for *Actinomyces p.* compared with *A. poolensis* and two strains of *A. scabies*. The following media were found most satisfactory for differentiating the fungi: Ashby's, synthetic, Endo, and nutrient agar. Ashby's medium proved to be most satisfactory for rapid vegetation and sporulating growth. The culture of *A. poolensis* and one of *A. scabies* were received from Waksman, while the other culture of *A. scabies* was isolated by Manns from scab lesions on white potatoes.

In addition to extensive cultural studies on differentiation, some work was undertaken to determine the response of these cultures to media to which pH indicators were added. Synthetic and Ashby's media were used, and to each 10 cc. of media there was added 1 cc. of indicator. The procedure followed was to inoculate the tube of melted agar and then add the indicator and pour into sterile culture plates. The first indicator used was brom cresol purple with a pH range of 5.2 to 6.8. Two uninoculated plates or controls of Ashby's medium were used for comparison and the plates were read after four days at a temperature of 33° C. With this indicator *Actinomyces p.* and *A. scabies* produced a change to yellow, indicating the acid reaction, while *A. poolensis* increased the intensity of the purple

color, showing the alkaline reaction. Thymol blue with a pH range of 1.2–2.8 and 8–9 was used with synthetic agar. In this series the plates were checked against indicators for final readings. *Actinomyces p.* and *A. scabies* showed 2.4 readings while *A. poolensis* and controls showed 2.8 readings. Brom cresol purple with a pH range of 5.2 to 6.8 with cultures on a synthetic medium showed that the controls and *A. poolensis* gave readings of 6.4 while *Actinomyces p.* and *A. scabies* gave readings of 5.4. These studies further supported the specific difference already established culturally between *A. poolensis, Actinomyces p.,* and *A. scabies.*

Temperature requirements are very important in relation to growth in culture as well as for infection studies of *Actinomyces p.* Room temperature is not favorable for optimum growth or infection. The optimum temperature range for this fungus was found to be between 30° and 37° C. At 52° C. very slight growth was made.

The first series of inoculation tests was conducted on slices of fleshy sweet-potato roots. Disease-free roots were selected and disinfected. Slices averaging ¼ in. in thickness were made and placed immediately on poured plates of plain agar. These plates were incubated for two days before being used for inoculation. The inoculum consisted of a single colony (¼–½ in. in diameter) transfer of *Actinomyces p.* from plates 10–14 days old. The series of tests were conducted under three ranges of temperatures, *viz.*, 20° C., 33° C., and 37° C. *Actinomyces p.* proved pathogenic under these conditions in developing a lesion restricted to the size of the inoculum within 48 hours at a temperature of 33° C., as illustrated in Plate III, A. Only a slight trace of infection occurred at room temperature. The lesion was typically black in appearance, and at the end of six days was conspicuously sunken and slightly larger than the inoculum. At 37° C. a slight, effused white growth of the fungus occurred, indicating sporulation. This method of inoculation was extended to include white potato, dahlia, carrot, beet, and turnip roots. Positive results were secured except in the case of carrot and dahlia. On white potato the character of the lesion was similar in appearance to that on sweet potato, whereas with beet and turnip the lesion was brown instead of black. The developing lesions were cut out and divided in half: one piece was used for reisolation work and the other killed and prepared for sectioning. This double check later proved that there were no contaminations.

The same method was used in further inoculations of sweet potato and white potato, but using in addition to *Actinomyces p.* the two strains of *A. scabies* and *A. poolensis.* The results secured from inoculations with *A. poolensis* and *A. scabies* received from Waksman were negative on both hosts, while *Actinomyces p.* and the strain of *A. scabies* isolated by Manns produced infection. This strain of *A. scabies* showed more evidence of being pathogenic on sweet potato than on white potato.

The growth of sprouts and root development were secured under sterile conditions by including the "eyes" of white potatoes and the growing points of sweet potatoes in the slices for use on poured plates of plain agar. Plates made up in this manner were kept at room temperature until roots or sprouts had grown enough to be inoculated. In this manner inoculations were made possible on such growing parts of the hosts as are not easily inoculated when the plants are grown in soil.

As soon as root points emerged from the fleshy roots of sweet potato they were inoculated with *Actinomyces p.*, and the resulting lesions were typical of those found under natural conditions. These results confirm field observations which always indicate that typical pox lesions on fleshy roots have their inception through rootlet infection. Growing rootlets an inch or more in length were next inoculated at the point of emergence from fleshy roots, which resulted in the development of typical pox lesions, as illustrated in Plate III, G. The resulting lesions secured by this method of inoculation on rootlets were restricted in growth to approximately the size of the inoculum. No extensive or progressive type of lesion occurred as a result of these inoculations. Inoculations on the uninjured surface or epidermis of fleshy sweet potato roots always gave negative results.

The next series of inoculations with *Actinomyces p.* was made at different points on rootlets which developed from slices of sweet potatoes planted on agar plates. The inoculum varied from a whole to half a colony and was placed directly on the rootlets growing on the surface of the plain agar plates. The resulting lesions were typically black in appearance and restricted in size to that of the inoculum. This typifies lesions on rootlets found under field conditions. Such rootlet infection under field conditions accounts for the sloughing of the root system and the general retardation of growth above ground. These rootlet lesions, when their pathological histology is studied, show much greater collapse of the tissue than is found in lesions on fleshy roots.

Inoculation of sprouts was next undertaken to parallel infections found under natural field conditions. Sweet potatoes were disinfected and bedded in sterile sand for propagation of sprouts. When sprouts reached 6–10 inches in growth, they were pulled. These sprouts were then placed in sterile cylindrical beakers of 1,000-cc. capacity with a layer of moistened cotton in the bottom and closed at the top with a plug of absorbent cotton. Most of the foliage growth protruded beyond the mouth of the beaker and cotton plug. All beakers were wrapped in black paper in order that light would not inhibit root development. Sprouts were carried under these conditions for three days before being inoculated. Development of sprout and root under these conditions occurred as normally as under bedded condi-

tions. In seven days, extensive root development of the controls occurred at a temperature of 33° C. and the layer of moistened cotton was ramified by the growing rootlets. Cultures of *Actinomyces p.* were prepared in 250-cc. flasks which contained 40 cc. of nutrient agar. When cultures were 14 days old, sterile glass beads and 125 cc. of sterile water were added. The flasks were shaken and the medium thoroughly broken up as in the form of a thin gel. The sprouts to be inoculated were then removed from the beakers and the entire zone of root development and part of the stem were immersed in this inoculum. These sprouts were replaced immediately in the sterile beakers and kept at a temperature of 38° C. The first series of sprouts was immersed for one minute, but it was later found that a three-minute immersion was more conducive to maximum development of lesions. This type of inoculation proved very successful in producing lesions similar to those found on sweet-potato sprouts under natural field conditions. Typical lesions appeared on various growing parts of the sprout within 7–10 days, the incubation period being longer than that following the previous types of inoculation. The origin of lesions appearing on the primary root of the sprouts within the zone of rootlet development always was a rootlet infection, as illustrated in Plate III, E and F. Typical, black, restricted lesions occurred at various points on the rootlets, as illustrated in Plate III, H. Lesions were removed for reisolation work and for study of their pathological histology. Consistent proof was secured that this phase of inoculation work was being conducted with a pure strain of fungus that was identical with the original *Actinomyces p.* Although the cultures used in these experiments were sporulated, it was not determined whether infection resulted from the spore or from the vegetative growth of the fungus.

A type of lesion occurred as a result of this method of inoculation that has not been previously described for sweet potatoes or observed under natural conditions. In instances in which the sprout was immersed in the inoculum above the zone of root development so as to include the green stem part, lesions developed which were different in appearance from those found on the root end of the sprout. These lesions first appeared as black pin points of infection, and, gradually enlarging more lengthwise than crosswise of the stem, grew to as much as 10 mm. in length and 4 mm. in width. Since this infection occurred above the zone of root growth, the infection can only be associated with entrance through the stomata. At maturity these lesions have a blackened margin and the center is brown and sunken. They are very slow in developing and do not appear until 4–7 days after lesions first appear on the roots. These lesions were cultured and prepared for cytological studies. The *Actinomyces p.* used in the inoculations was also recovered in pure condition.

Another series of experiments was established by inoculating sprouts as above described with *A. poolensis* and *A. scabies,* the latter fungus being from the culture isolated by Manns. Results were negative with both fungi. The failure of *A. scabies* to produce infection under these conditions may be attributed to improper technique or to the fact that it is weakly pathogenic on sweet potato sprouts. Sprouts inoculated under these conditions with *Actinomyces p.* and *A. scabies* always showed a retarded growth and root development compared with controls, while inoculation with *A. poolensis* had no such effect. This reaction tends to indicate that a toxin or some product of the fungous metabolism retarded growth. A preliminary test was made under controlled conditions of the effect of the filtrate from cultures of *A. scabies* on root growth of white potatoes, and the results of tissue collapse were very positive. Further detailed work should be conducted, however, before the preliminary results are reported.

The histological preparations of the diseased lesions on sweet potatoes were found to be most easily differentiated with the iron alum haemotoxylin stain. The fungus observed in all lesions prepared for study resembled that used in all inoculations and studied in pure culture. The development and effect of the fungus in the host tissue are identical in both the natural and the artificially produced lesions. The fungus appears to be inter- as well as intra-cellular. It is most conspicuous within the host cells and usually is evident as a mass in the area of the necrotic lesion. The cells making up the lesion usually retain their identity but are devoid of their cellular contents. The parasitism is not associated with a complete collapse of all the cellular tissue making up the lesion, as is indicated by the macroscopic appearance of the lesion.

DISCUSSION

Halstead (2) was apparently the first to associate a fungus with sweet-potato pox, naming the causal organism *Acrocystis batatas* E. and Hals. Elliott (1) interprets the illustrations of Halstead as apparently various stages in the life history of the plasmodium *Cystospora batata,* which he described as the cause of pox. Taubenhaus (13) states that "the genus *Acrocystis,* as originally described by Halstead, is not valid and is non-existent. The fungus *Acrocystis batatas* was mistaken by Halstead for a myxomycete which Elliott has named *Cystospora batata,* the true cause of pox (soil rot)." After making extensive studies of various prepared materials of diseased roots and fleshy roots of sweet potatoes, the writer realizes how easily various foreign bodies were mistaken by these early investigators for stages in the life history of pathogenes. Species of *Rhizoctonia, Pythium,* and undetermined Phycomycetes are often found associated with different disease symptoms on sweet potato, in addition to the fungous diseases known as black rot and soil stain. Nematodes are not uncommon in some of the mature pox lesions and are also found in the rootlets. Nema-

todes in various stages of their life history are found within the tissue of rootlets and fleshy roots as contamination of lesions. Histological preparations of the pox lesion with infestation of nematodes naturally show various types of sections through young and mature nematodes as well as egg masses. In many instances such sections are not unlike certain illustrations and descriptions given for *Acrocystis batatas* and *Cystospora batata*. Poole and Schmidt (12) have given careful study to the nematode disease of sweet potatoes. It is stated that "on susceptible varieties the symptoms were so-called knots and scabs on stems, roots and potatoes. On the susceptible varieties malformation was produced; scabby areas, followed by pit-like rots, were prominent; but raised pustule-like areas were not very abundant on potatoes." Their illustrations of scab and pit areas on sprouting points are not unlike symptoms of pox. In the case of small potatoes and roots in which female nematodes were abundant, the effect is not unlike the initial symptoms of pox lesions. Their studies further indicate the possibilities of confusion in studying the pox disease, which may account in part for some of the descriptions reported by the earlier investigators.

The fungus *Actinomyces poolensis* was used for comparison, because Taubenhaus (13) has reported this fungus as a "superficial wound parasite, usually found following the pox spots produced by *Cystospora batata*. . . . Inoculation experiments with pure cultures of this organism showed that it is capable of producing a spot which, although not resembling pox, may penetrate equally deep in the host tissue."

The results of inoculations showed that *Actinomyces p.* and *A. scabies* were pathogenic on cut slices of sweet potato and white potato as well as on rootlets from sprouted pieces of their hosts developed under sterile conditions. When inoculation of sweet potato sprouts was undertaken with *A. scabies* isolated by Manns from white potato by immersion in an inoculum of macerated colonies, the results were negative on the roots as well as on the stem. It is possible the period of immersion in the inoculum was too short. This method should be as effective as direct inoculation with colonies of the fungus. The infection of the roots and stems of sweet potato sprouts with *Actinomyces p.* is similar to the infection of stems and stolons of white potatoes by *A. scabies* as reported by Jones *et al.* (3). This type of infection on these two hosts is associated with an initial crippling of the root system, which is indicated by reduced yields where heavy infection occurred. However, root infection of white potatoes by *A. scabies* has not been demonstrated by investigators in this country as an important phase of the disease. Millard and Burr (10) demonstrated root infection of white potato with *A. scabies* in Great Britain. They report that scab rarely appears to exert any adverse effect on yield but consider that reduction of yields in America may be accounted for by root infection.

Actinomyces p. and *A. scabies* are easily differentiated from a cultural standpoint, and were shown by cross inoculations to be pathogenic for sweet potato and white potato, respectively. Millard and Burr (10) have established 11 species of *Actinomyces* that were found to produce scab on white potatoes. Their work, as well as that of Wollenweber (14), further shows the possible confusion that may exist in working with this group of *Actinomyces.* Because of this fact, it would seem most feasible to determine further possible relations between other species already described and this Actinomycete on sweet potatoes.

It is significant that there is a parallel response between the scab of white potatoes and the pox of sweet potatoes to sulphur as a fungicide. The effect of acid soil conditions is an inhibiting factor in the prevalence of these two diseases. It has been proved in field experiments that the amount of soil acidity tolerated is much greater for the sweet potato than for the white potato.

SUMMARY

Studies of the pox or soil rot in sweet potatoes have failed to produce evidence that it is caused by either the fungus *Acrocystis batatas* E. and Hals. or the slime mold *Cystospora batata* Elliott.

A species of *Actinomyces,* for convenience referred to as *Actinomyces p.,* has been isolated from pox lesions on sweet potatoes. This fungus has been found pathogenic on fleshy roots and cut slices of sweet potato, on emerging root-points and rootlets from fleshy roots as well as on the primary root, and on rootlets and stems of sweet-potato sprouts. Resulting lesions are similar to pox lesions occurring on this host under field conditions. Cytological study has shown this same fungus to be present in naturally as well as artificially produced pox lesions on sweet potato.

Actinomyces p. was found pathogenic on cut slices of white potato, beet, and turnip but gave negative results on carrot and dahlia.

Actinomyces p. and *A scabies* were shown to be different from a cultural standpoint, but were pathogenic on cut slices as well as rootlets of sweet potato and white potato.

Cultural and inoculation studies demonstrated that *Actinomyces p.* is different from *A. poolensis,* since the latter fungus was not found to be pathogenic on sweet or white potatoes.

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EXPLANATION OF PLATE

A. Sweet potato slices in sterile agar plates which have been inoculated with *Actinomyces p.* and kept at different temperatures for varying periods. Reading left to right: 33° C. for 7 days, 33° C. for 4 days, and room temperature (20° C.) for 4 days.

B. White potato slices in sterile agar plates which have been inoculated with *Actinomyces p.* and kept at temperatures as described in A for sweet potatoes.

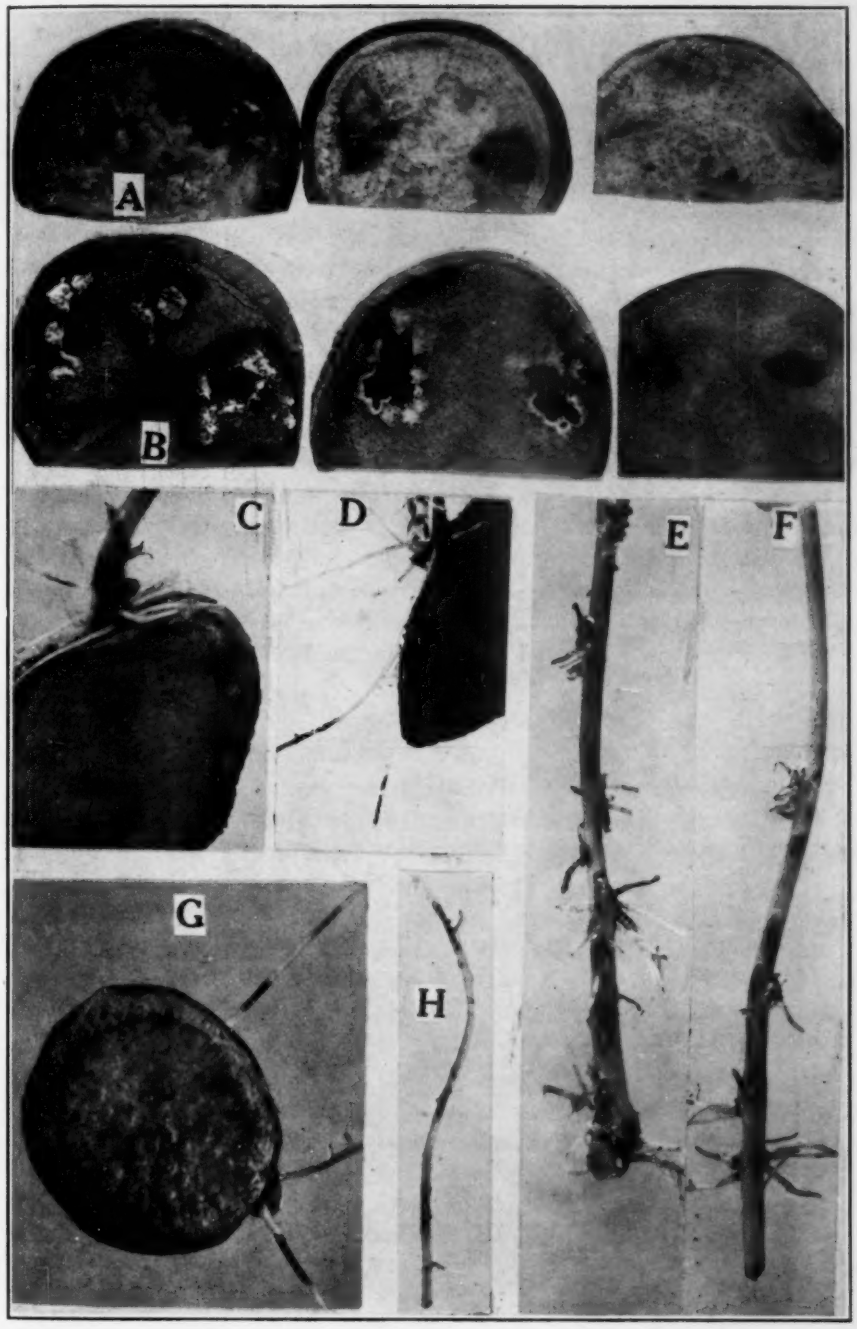
C. Blackened lesions on the base of the sprout and rootlet of white potato inoculated directly with *Actinomyces p.* Inoculation made Feb. 16 and photographed Feb. 22, 1927. Sprouted at room temperature in sterile agar plates.

D. Blackened lesions on rootlets of white potato inoculated directly with *Actinomyces scabies.* Inoculated Feb. 16, 1927, and kept at room temperature for 6 days. Sprouted at room temperature in sterile agar plate.

E and F. Sweet potato sprouts grown under sterile conditions and immersed for three minutes in an inoculum consisting of macerated colonies of *Actinomyces p.* (see text). The sprouts were then removed and placed in sterile beakers with moistened cotton for a substratum. Beakers were plugged at mouths with cotton. The sprout illustrated in fig. E was inoculated Mar. 14 and photographed April 4, 1927; the sprout illustrated in fig. F was inoculated Mar. 25 and photographed Apr. 4, 1927. The secondary root system had been cut off (before photographing) in order to illustrate the primary root lesions.

G. Blackened lesions on rootlets of sweet potato inoculated directly with *Actinomyces p.* sprouted at room temperature in sterile agar plate. Inoculations made Jan. 28 and photographed Jan. 31, 1927.

H. Typical blackened lesions on rootlet cut off of sprout that was immersed for three minutes in an inoculum consisting of macerated colonies of *Actinomyces p.* The sprout from which this rootlet was removed is shown in fig. F.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MONOGRAPH OF THE GENUS PESTALOTIA DE NOTARIS[1,2]
PART I

E. F. GUBA

INTRODUCTION

My interest was directed to the genus *Pestalotia* in the autumn of 1922 by Dr. F. L. Stevens, who had returned to the University of Illinois after several months of botanizing in Hawaii. His collection of fungi contained several specimens of *Pestalotia* which he assigned to me for determination. The volumes of Saccardo's "Sylloge Fungorum" and an excellent botanical library giving access to other compilations and to type descriptions were available for this purpose; nevertheless the specimens could not be accurately identified. The difficulty lay with the indefinite, inaccurate, and carelessly prepared type descriptions. As a result, serious errors in classification have crept into the literature. The general lack of understanding of the species has emphasized the urgent need of their critical study. This first contribution presents a critical study of 46 described species, of which 30 are retained as distinct. It also presents a review of our knowledge of their biologic relationships.

HISTORY OF THE GENUS

The genus *Pestalotia* was created in 1839 by Dr. J. de Notaris (57, p. 80–81) and named in honor of his friend Dr. Fortunato Pestalozza. It was established on the basis of the fungus *Pestalotia pezizoides* de Not. occurring on dead canes of *Vitis vinifera*. The genus was described as follows:

"Sporidia pedicellata pluriseptata, articulo supremo in setas divergentes soluto, infero mutico, vel uni-bi setosa, stromata gelatinoso juncta, ex epidermide erumpentia, demum effusa. Fungilli sub-epidermide ramorum emortuorum nascentes demum liberati, acervula convexa tuberculiformia, in sicco depressa collapsa, sub pezizoidea, sporidia atroinquinantibus."

The fruiting structure was regarded as an acervulus (Plate IV, A). Being seated on a stroma above the epidermis and resembling the outline of an apothecium, de Notaris assigned to it the very appropriate name

[1] The writer is gratefully indebted to Dr. C. W. Dodge, of Harvard University, for helpful suggestions and criticisms in the preparation of this manuscript and for permission to use the facilities of the Farlow Library and Herbarium.

[2] Printed with the permission of the Director of the Massachusetts Agricultural Experiment Station.

Pestalotia pezizoides. The description and illustration of the type of *Pestalotia pezizoides* were prepared carefully enough and sufficiently in detail to offer no chance of error in the identification of this species. The fungus is widely distributed and has been collected generally in Europe and North America, where it is richly represented in exsiccati collections and herbaria.

The first addition to the genus was made by Desmazières in 1840 as *Pestalotia guepini* Desm. on Camellia leaves. This species has 5-celled spores, but is similar to the type of the genus in that the end cells of the spores are hyaline and the apical cell is setulate. The fruiting structure, however, is typical of an acervulus (Plate IV, C.) Desmazières' description proved so indefinite that this species has never been clearly understood. Being the earliest of the 5-celled forms to be reported, its name received cosmopolitan application, a practice which later resulted in innumerable errors in classification. *Pestalotia funerea* Desm., created in 1843, shared a similar fate.

From 1840 to 1880 many new species were added to the genus, the majority of which have 5-celled spores. Saccardo (Syll. Fung. **3**: 784. 1884) created the subgenus *Eu-Pestalozzia* for those forms having spores with hyaline end cells, colored median cells, and two or more apiculate setae; *Monochaetia* for those with similar spores but uni-setulate; and *Pestalozzina* for forms with totally hyaline conidia. This arrangement was accepted by Voglino (87) in his compilation of the genus which appeared in 1885. He recognized 89 species, of which 62 belonged to the sub-genus *Eu-Pestalozzia* and 19 to *Monochaetia.* Saccardo (Syll. Fung. **11**: 580. 1895) elevated the sub-genus *Pestalozzina* to generic rank and Allescher (Rabenhorst, Kryptog. Fl. I. **7**: 665. 1902) did the same for *Monochaetia,* while *Pestalotia* was restricted to the sub-genus *Eu-Pestalozzia.* Höhnel (39) transferred *Monochaetia* to *Hyaloceras.* Diedicke (24) approved of this transfer. Höhnel (40) later, in his system of the Fungi Imperfecti, disregarded *Hyaloceras* and retained *Monochaetia.*

Klebahn (43) divided the genus *Pestalotia sensu strictiore* into sections determined by the number of spore cells as follows:

Quadriloculatae—4-celled spores, two of the cells colored and two hyaline; *e.g., P. stevensonii.*

Quinqueloculatae—5-celled spores, three cells colored and two hyaline; *e.g., P. funerea* and *P. versicolor.*

Sexloculatae—6-celled spores, four colored and two hyaline; *e.g., P. pezizoides.*

This arrangement has been adopted in the text of this paper.

A large amount of literature of a controversial nature exists concerning the pathogenicity of forms of *Pestalotia.* Wenner (89), Bernard (3, 4, 5, 6,

7), Spaulding (74), Brown (12), Tubeuf (82, 83, 84), Laubert (50), Böhm (10), Hennings (38), and others have attributed pathogenic capabilities to certain species, while Doyer (25), Münch (56), Fischer (28) and others regarded them as saprophytes. Doyer (25) denied the pathogenicity of every species reported to be parasitic but her evidence is inconclusive. It appears from a review of the literature that the parasitic nature of many forms has been based upon the constant presence of the fungus on diseased parts of plants rather than on positive evidence of infection obtained by experimental methods. In her selection of material for infection purposes and for determining pathogenic relationships, Doyer disregarded the probable restriction of forms to their hosts. Her failure to infect plants with forms having no biologic relationship to them offers no reasonable basis for accepting her statement of the non-parasitic nature of such species.

La Rue and Bartlett (49) recognized 35 distinct strains within the nominal species of a form identified incorrectly as *P. guepini* Desm. The 35 strains were capable of precise definition along morphological lines into 14 groups. These were obtained from coconut palm, oil palm, betel-nut palm, rubber, and tea, and were not confined to any particular host. La Rue (48) showed that selection is totally ineffective in establishing distinct lines within pure strains of *P. guepini*. Aberrant spores produced normal progeny, while mutations giving rise to lines significantly different from parent lines rarely occurred. These workers accepted the view that the descriptions of the nominal species of *Pestalotia* are generally in no degree diagnostic. They regarded forms obtained from rubber, coconut, tea, oil palm, and betel-nut as the same species. The refined biometrical methods which La Rue and Bartlett have emphasized in arriving at their conclusions seem unnecessary for the classification of species.

Doyer emphasized cultural characters and biometric differences in distinguishing forms, but the study of types is ignored and her knowledge of species is based on existing literature and the carelessly prepared type descriptions. Reports of the occurrence of the same forms on a wide variety of plants have been accepted too freely and her incorrect designation of forms is open to criticism.

The spelling of the generic name received modification from *Pestalotia* to *Pestalozzia* since its inception. Corda (Icones Fungorum **5**: 34: 1842) apparently was the first to use the modified spelling. Fairman (27) has shown that *Pestalotia* is both as correct and euphonious as *Pestalozzia*. Such being the case, the original spelling will be used.

MORPHOLOGY

Fruiting Pustules

It is recognized that the genus *Pestalotia* has no typical fruiting structure. Wolf (90) found true pycnidial structures of *P. uvicola* Speg. on

rotted grape berries. The spores were formed within a mass of false tissue and emitted through a narrow ostiole. Wolf believed that the generic description should be modified to include range of types from the flat surface on which spores are formed without pseudoparenchyma to those which form well defined pycnidia. Leininger (52) obtained four types of fructification among forms from *Echeveria* and *Mesembryanthemum* which he called *P. palmarum*. These are as follows: (*1*) Free spores on mycelial threads in liquid media; (*2*) spores on open acervuli and on a loose parenchymatic stroma in liquid media; (*3*) spores in acervuli covered by pseudoparenchyma on solid media; (*4*) spores in pycnidia on solid media.

Leininger found spore bodies in nature with a covering membrane and cavities completely enclosed with hyphae which in basal portions formed a more or less distinct pseudoparenchyma. Klebahn (42), Lagerberg (47), Sartory and Bainier (2, 68), Fischer (28), Doyer (25), and Bertus (8, 9) obtained in culture one or more types of fructification reported by Leininger. Archer's (1) study of the development of the spore bodies of *P. guepini* and *P. palmarum* in culture showed that they originate from a true pycnidium and that cavity formation is like that in the genus *Sphaeropsis*. Klebahn stated that the genus was incorrectly placed in the Melanconiaceae and that its inclusion among the Sphaerioidaceae-Phaeophragmiae close to the genus *Hendersonia* would be more proper. Previous to Saccardo's "Sylloge Fungorum" the fruiting structure of *Pestalotia* was considered a receptacle [Bornorden (11, p. 220 and 228), Frank (31, p. 404 and 32, p. 440), Corda (19, p. 141), Léveillé (53)], in consequence of which the genus was placed with genera many of which are now listed among the Sphaeropsidales. Notaris and Desmazières believed that a receptacle was lacking, while both Montagne and Léveillé observed a covering membrane.

Elenkin and Ohl (26, pp. 94–100) claimed that *P. malorum* E. & Ohl as well as *P. hartigii, P. disseminata,* etc., because of the formation of pseudopycnidia, would be more properly placed in a new genus *Pseudopestalozzia* in the group Pseudosphaerioidaceae Höhnel. Höhnel (40) disposed of the genus in the Gymnostromaceae. Both Höhnel (40) and Potebnia (64) have devised new schemes for classifying the genera of the Fungi Imperfecti, but Gäumann (33, pp. 585–587) and Doyer (25) consider Saccardo's system preferable, as modified by Lindau (Engler and Prantl. Die Natürlichen Pflanzenfamilien. Pilze I. **1**: 347–523, 1900), Allescher (Rabenhorst's Krypt. Fl. Pilze. I, **6**: 1–1016, 1901; and **7**: 1–1072, 1903), and Diedicke (Kryp. Fl. Mark. Brand. IX. **7**: 1–962, 1915). Since the fruiting structure of *Pestalotia* may be either an acervulus or a pycnidium the genus may belong to both the Sphaeropsidales and Melanconiales. A simple method of classification would be to place together all setulate spore forms now in the Sphaeropsidales and Melanconiales and to arrange the genera according to form, color, and structure of the spores.

Conidia

The genus is familiar to students wholly by the character of the conidia. These are fusiform in shape, 3-, 4-, or 5-septate with guttulate, colored median cells and hyaline end cells. The spore is supported at the base of the inferior hyaline cell usually by a simple stalk. The apical, hyaline cell is crowned with a crest of 2 or more simple or branched setae. Specimens of *P. pezizoides* in the group Sexloculatae Klebahn reveal an occasional spore with 6 setae. In the 3- and 4-septate spore groups, abnormalities in the number of septa occur. Likewise a variable number of setae on spores from the same specimens may occur, but no specimens have come to my attention whose disposition in either *Monochaetia* or *Pestalotia* required the least hesitation. The elevation of *Monochaetia* and *Eu-Pestalozzia* to generic rank was done in recognition of this fact. Doyer's (25) opinion that they should be combined as one genus is incorrect. The presence of aberrant spores should offer no confusion in classification as they are always negligible in number.

The color intensities of the median cells of the spores have been used as a character for differentiating species. According to Saccardo's "Chromotaxia," the median cells may be olivaceous, umber, or fuliginous. All the median cells may be colored alike or the upper two may be darker than the lower. Doyer arranged the 5-celled spore forms into three groups with spore-color characters as follows: (*1*) *funerea*—three dark middle cells, the lowest the brightest; (*2*) *guepini*—three bright olive colored cells, equally colored; (*3*) *versicolor*—dark band at septa dividing upper two colored cells, lowermost colored cell the brightest. Color contrasts of the median cells, therefore, are considered useful in differentiating species.

The setae never taper to a point, but knobbed setae have been noted by Voglino (87) for *P. clavata* Cke. & Ellis, by Sawada (69), Butler (13) and Bertus (8, 9) for *P. theae* Saw., and by Bertus for *P. palmarum* Cke., and I have found them typical of other species. Lacroix (46) reported that, in the case of *P. funerea* Desm. and *P. choenostroma* Lacr., the rudimentary setae terminate by a slight swelling which later disappears and that the walls of the filaments never taper but remain parallel. However, knobbed setae are constant with certain species. Such species usually have knobbed pedicels. Infrequently one or more of the setae originate at the margin of the uppermost septa of the spore. Graves (35) found such a condition existing among spores of a species which he designated *P. funerea* Desm., and Voglino (87) reported this characeter for *P. montellica* Sacc. & Vogl. Branched setae are typical of many species, notably *P. guepini* Desm. and *P. pezizoides* de Notaris. These spore characters are extremely useful in differentiating species.

A close relation exists between *Pestalotia* and *Hendersonia*, for according to Lagerberg (47) cultures of *P. hartigii* yielded some conidia similar to those of *Hendersonia* and some resembling those of *Monochaetia* and *Coryneum*. According to Massee (54), *P. guepini* cultivated on bread produced conidia without setae exactly like those of *Hendersonia theicola* Cke., but when conidia of the latter were transferred again to prune decoction only Pestalotia conidia were produced. These he regarded as abnormal forms of *P. guepini*. On the other hand Speshnev (75) failed to obtain setulate conidia in cultures of *Hendersonia theicola* therefore concluding that *P. guepini* and *Hendersonia theicola* were distinct. Crié (20) found setulate spores resembling *Pestalotia* among the conidia of certain forms of *Hendersonia*. Spores resembling those of *Pestalotia* and *Diplodia* were found in the same receptacle. These variations in spore form suggest that the species are in a plastic condition, but La Rue (48) found that selections according to progeny and visible characters for several generations failed to establish any differences and that selection was ineffective in establishing distinct lines within pure strains. Mutations giving rise to lines significantly different from parent lines infrequently occurred.

Other Spore Forms

Sartory and Bainier (2, 68), Wenner (89), and Doyer (25) found chlamydospores in cultures. The spores are borne in long chains which finally fall apart. The cells of these spores are capable of germinating and again forming mycelium and conidia. Wenner was the first to show the existence of a second spore form in the genus. According to Wenner, chlamydospores may arise in either of two ways as follows: (1) any cell or several cells together in a hypha may round out and become fairly thick-walled spores; (2) the entire distal portion of a hypha may form consecutive, short, cylindrical spores with fairly thick walls. These, according to Wenner (89), are similar to oidia.

Höhnel (41) reported both *Ceratostoma vitis* Fuckel and *P. truncata* Lév. on *Humulus lupulus* in Krieger, Fung. Sax. 580 and assumed that the former was the ascigerous stage of the latter. This is the only report of the ascigerous stage of a *Pestalotia*. This connection seems very doubtful, especially in the absence of study of either form in pure culture. Species of *Pestalotia* existing as saprophytes on dead, decaying parts of plants are frequently associated with other fungi. The latter cannot be considered related because of mere association.

Taxonomy

PESTALOTIA de Notaris, Microm. Ital., Dec. II. Mem. R. Accad. Sci. Torino II. **3**: 80–81. 1839.

Fruiting bodies black, carbonaceous, either acervuli with or without stromata or pycnidia. Spores fusiform, 4- to 6-celled of which the exterior

cells are hyaline and the intermediate cells dark-colored, guttulate; superior hyaline cell with a crest of two to several simple or branched setae, inferior hyaline cell supported by a simple or branched pedicel.

Section Sexloculatae Klebahn, Myc. Centralbl. **4**: 19. 1914.

Acervuli seated on a stroma composed of erect, septate, branching or simple filaments, pezizoid. Spores fusiform, 6-celled, interior cells colored, exterior hyaline; superior cell crowned with a crest of two or more simple or branched setae, inferior cell tapering into a branched stalk. The type is *Pestalotia pezizoides* de Notaris on dead canes of *Vitis vinifera*, Milan, Italy.

1. PESTALOTIA PEZIZOIDES de Notaris, Microm. Ital. Dec. II. Mem. R. Accad. Sci. Torino II. **3**: 80–81. 1839; Pirotta, Arch. Trienn. Lab. Bot. Critt. Pavia **2**: 196–198. 1878; Sacc. Syll. Fung. **3**: 789. 1883.

   P. compacta B. & C., Grevillea **2**: 155. 1874; Sacc. Syll. Fung. **3**: 795. 1884.

   P. ramosa D'Almeida, Rev. Agr. Soc. Sci. Agron. Portugal **1**: 23–26. 1903; Sacc. Syll. Fung. **18**: 481. 1906.

Fruiting bodies definite, scattered or gregarious, often confluent, erumpent, borne on a thick gelatinous stroma, discoid, pezizoid or subglobose, 225–600 × 100–300 μ, protruding well above the epidermis (Fig. 1, b).

Spores long fusoid, equilateral, sometimes slightly curved, tapering at the ends, 6-celled, 27–36 × 6–9 μ; intermediate cells olivaceous, slightly constricted at the septa, 19–26 μ long; setae short, 2 to 5, branched or simple, 6–18 μ, recurved, rarely with a solitary branched seta and 12–31 μ; pedicel simple or forked, 2–10 μ, sometimes 5–22 μ, slender (Plate —, A and B).

On canes of *Vitis vinifera*, *V. riparia* and related species.

Specimens examined: Michener Fungi. **19**: 82, 2790, N. Garden, C. Co., Pa., sub *P. compacta* B. & C., in Myc. Coll. U. S. D. A., and similar specimens in Curtis Herbarium, *6030*, at Harvard and Berkeley's Herbarium at Kew, wrongly cited on stems of *Humulus lupulus;* Ellis, No. Amer. Fung. *35*, on grape vines; Ravenel, Fung. Amer. Ex. *256* on twigs of Vitis, Aiken, So. Carolina, 1877; Barth., Fung. Col. *1631*, on dead *Vitis riparia*, Rooks Co., Kans., Nov. 15, 1901 and March, 1897; on *Vitis sp.*, Marshall Hall, Md., V. K. Charles, 7–6–06, Myc. Coll. U. S. D. A. sub *P. ramosa* D'Almeida.

The acervulus is supported by a dense thick stroma which arises above the epidermis and in habit resembles the apothecium of a *Peziza*. The spores are exposed on flat or concave stromatic cushions. They are 6-celled and possess other characters different from our present conceptions of the genus. Pirotta published a very good account of the fungus. Cooke

(Nuovo Giorn. Bot. Ital. **10**: 27. 1878) regarded *Hendersonia longipes* B. & C. synonymous with *P. pezizoides*. According to the type description of *H. longipes* B. & C., this form has 3-septate spores and no setae.

In 1903 D'Almeida created the species *P. ramosa* on canes of *Vitis vinifera*, Chamusca, Portugal. The figures accompanying the description show clearly that the fungus is identical with *P. pezizoides* de Notaris. Duplication of this species was due apparently to confusion in the literature regarding the number of septa in the spores. De Notaris specified and figured 5-septate spores although he allowed for an exception when he stated "sporidia . . . plerumque 5 septata." Saccardo (Syll. Fung. **3**: 789. 1883) and Allescher (Rabenhorst, Kryptog. Fl. Deutschl. I. **7**: 693. 1902) stated that *P. pezizoides* has 5-celled conidia. Voglino (87) regarded it as a 4- to 5-septate form. Desmazieres (Ann. Sci. Nat. Bot. II. **19**: 336. 1843) mentioned 5 septa. Klebahn (43) observed 6-celled conidia in specimens of *P. pezizoides* in Barth., Fung. Col. *1631*. My study of many exsiccati specimens confirms the 6-celled character of the spores of this species.

The name *P. compacta* B. & C. was apparently intended for another species, for the type description by Berkeley mentioned 3-septate spores and the source of the material as South Carolina. The original material was acquired by Michener in Pennsylvania. Michener gave specimens to Curtis in South Carolina and Curtis shared his material with Berkeley. The pencil drawing of the fungus accompanying the type material from Kew, presumably by Berkeley, shows 5-septate spores. Either an error was committed in the designation of the host by the collector or Michener confused this material with something else. Dr. E. C. Jeffrey of Harvard University, to whom material was submitted, reported that the host is undoubtedly a species of *Vitis*, not *Humulus lupulus*. The fungus is identical with *P. pezizoides*.

Section Quinqueloculatae Klebahn, Myc. Centralbl. **4**: 3. 1914.

Pustules subepidermal, erumpent, typical of acervuli or pycnidia; spores fusiform, 5-celled, interior cells colored, exterior hyaline: superior cell with a crest of two to several setae: inferior cell supported by a simple pedicel. The type is *Pestalotia guepini* Desm. on leaves of *Camellia japonica*, Angers, France.

2. PESTALOTIA GUEPINI Desm. Ann. Sci. Nat. Bot. II. **13**: 182–184. 1840. pl. 4, fig. 1–3; Sacc. Syll. Fung. **3**: 794. 1884.
   Coryneum camelliae Massee. Grevillea **20**: 8. 1891. Kew, Bul. Misc. Inform. 138: 106–109. 1898.
   Pestalotia karstenii Sacc. & Syd. Sacc. Syll. Fung. 14: 1030. 1899.

P. inquinans Karst. Hedwigia **30**: 301. 1891; Sacc. Syll. Fung. **10**: 487. 1892; not Cke. & Harkn. Grev. **12**: 94. 1884.

Spots about 1.5 cm. in diameter or variable in area and shape, ash gray on the upper surface, sometimes with a definite dark or reddish brown border; frequently the dead areas are confined to the margins of the leaves, or the entire leaf may be colored brown and covered with the fungus (Fig. 1, a).

Fruiting bodies on grayish papery spots or scattered over the entire surface of the leaf, punctiform, black, 90–170 μ diam., numerous, amphigenous,

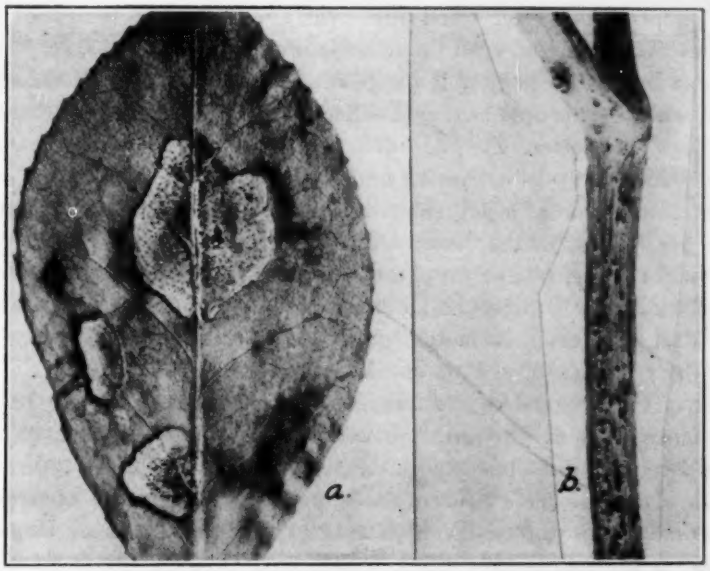

FIG. 1. a. *P. guepini* Desm. on leaf of *Camellia japonica*.
b. *P. pezizoides* de Notaris on cane of *Vitis vinifera*.

globose-lenticular, rupturing the epidermis, the black contents spreading over the surface of the leaf.

Spores erect, 14–21 μ, elliptic-fusoid, 5-celled, hardly constricted at the septa; end cells hyaline, short-conic, not tapering, sometimes rather inconspicuous; median cells olivaceous, guttulate, 10–14 × 5.5–6.6 μ; setae 1, 2, 3, or rarely 4, sometimes with a small inconspicuous knob at the apex, 10–24 μ, divergent, commonly branched; pedicel short, straight, often deciduous (Plate IV, C and D).

On stems and leaves of *Camellia japonica*, usually associated with pale colored spots (Fig. 1, a).

Specimens examined: (on leaves), Desmazières, Pl. Crypt. de France **22**: 1084, Angers, France, Guepin; Roumeguère, Fung. Sel. Gall. Ex. *315*,

Hort. Lotharingiae and Alsatiae; Thüm., Fl. Aust. Ex. *1070*, Bohemia, 1873; Ellis, No. Amer. Fung. *256*, Westchester, Pa., in greenhouse, G. Martin, Sept. 1880; Briosi and Cavara, I Funghi Par. VI, *150*, Pavia, 1890; Rab., Fung. Eur. Ex. *2352*, in Hort. Bot. Neapolitano, Cesati; Tuskegee Inst. Alabama, 3–7–25, G. W. Carver; Alabama, 3–17–12, F. A. Wolf, Myc. Coll., U. S. D. A. sub *P. inquinans* Cke. and Hark.; Washington, D. C., in greenhouse, 5–20–07, W. A. Orton, Myc. Coll., U. S. D. A. sub *P. camelliae* Pass.; Pass Christian, Mississippi, 1–29–16, E. Martin, Myc. Coll., U. S. D. A.; Herb. J. A. Stevenson *1304*, Oneco, Fla. 2–10–21; Washington Insp. House, P. I. *5099* U. S. D. A., Nantes, France; Cooke F. Brit. Ex. *625*, 2nd Ed.

P. guepini has been cited incorrectly on a wide range of plants. Speshnev (75) reported it on *Camellia japonica, Thea, Citrus, Magnolia* and *Rhododendron* in the region of the Black Sea. The form on *Thea* which he studied was described as having 4-celled spores. Saccardo (Syll. Fung. **3**: 794. 1884) reported *P. guepini* on *Camellia, Rhododendron, Citrus, Lagerstroemia, Magnolia, Smilax* and *Amygdalus*. La Rue's (48) concept of *P. guepini* is that of a polymorphic fungus containing many strains causing the gray blight of tea, leaf spot of coconut, betel-nut palm, African oil palm, para rubber, and other plants, but this view is not acceptable in the light of studies by Butler (13), Bertus (8, 9), and Archer (1), which have shown that the forms from tea, palm, and *Camellia* are distinct species. According to my studies, *P. guepini* is restricted to *Camellia*.

Massee (54) regarded *Hendersonia theicola* Cke. (Grev. **1**: 90. 1872) synonymous with *P. guepini*. Massee, like Speshnev, illustrated and described 4-celled spores but his illustration of the leaf spot is typical of the Camellia disease. He cultured the fungus from *Camellia* on bread and found some spores wholly devoid of bristles, similar to those of *Hendersonia theicola* on tea leaves; but when transferred to plum juice gelatin these spores produced normal conidia. Speshnev disagreed with Massee because cultures of this *Hendersonia* failed to obtain *Pestalotia* conidia. Massee also failed to offer proof that *Pestalozzina camelliae* Pass. (Rev. Myc. **9**: 146. 1887) is identical with *P. guepini* Desm. The type description is distinctly that of a *Pestalozzina*.

The type specimen of *P. guepini* Desm. in Desmazières Pl. Crypt. de France **22**: 1084, was inaccurately described and illustrated but the characters of the original specimens agree with the description of *P. inquinans* Karsten (Hedwigia **30**: 301. 1891) = *P. karstenii* Sacc. & Syd. (Sacc. Syll. Fung. **14**: 1030. 1899) occurring on living leaves of *Camellia*. The name *P. karstenii* was substituted for *P. inquinans* to avoid confusion with another *P. inquinans* Cke. & Hark. (Grev. **12**: 94. 1884) on dead leaves of *Eucalyptus*.

According to Klebahn (43) the conidia of *P. guepini* in Roumeguère, Fung. Sel. Gall. Ex. *315*, do not agree with those of the type illustrated by Desmazières. This specimen has short elliptic-fusoid spores occasionally with forked setae in contrast to the long, narrow, fusoid spores with simple setae illustrated by Desmazières. Massee (54) illustrated spores of *P. guepini* with 3–4 simple setae but with only 3 septa. Voglino (87) figured spores of *P. guepini* similar to Desmazières' illustrations. His description of the fungus is accompanied by reference to the following exsiccati; Rab. Fung. Eur. Ex. *2352*, on leaves of *Camellia japonica:* Thüm. Fung. Aust. Ex. *1070*, on leaves of *Camellia japonica:* Ravenel, Fung. Amer. Ex. *553*, on leaves of Persimmon and Cooke Fung. Brit. Ex. *625*, 2nd Ed. on leaves of *Camellia japonica*. Ravenel, Fung. Amer. Ex. *553*, on persimmon leaves labelled *P. guepini* is a different species.

Karsten's description of the setae of *P. karstenii* Sacc. & Syd. (*P. inquinans Karsten*) is as follows: "Ciliis apicalibus 2, rarissme 1 vel partitione 3, divergentibus, sub-aequalibus, hyalinis, 15–35 μ longis." Other differences stated here are: "*A Pestalozzia guepini* Desm. Camelliam incolente, acervulis, macula pallescente insidentibus, mox superficialibus nex non cilus paucioribus discrepat." Allescher (Rabenhorst, Kryptog. Fl. Deutschl. I. **7**: 685. 1902) and Klebahn (Myc. Centralbl. **4**: 7–9. 1914) regarded *P. karstenii* a synonym, and my study of the type confirms this view. Desmazières figured spores of the type with pointed setae, but Lacroix (46) never observed such setae in his studies in spore development, and I have never encountered them among any forms. Since the fungus was carelessly described, Karsten was unable to reconcile his fungus with the description, whereupon he created another species.

Massee (54) proved by artificial inoculation that *P. guepini* is a parasite and the cause of a common leaf spot of *Camellia*.

3. **Pestalotia vaccinii** (Shear) Guba, n. sp.
   P. guepini Desm., var. *vaccinii* Shear, Bul. Torrey Bot. Club **29**: 456–457. 1902.

Acervuli hypophyllous, subepidermal then erumpent, black, coalescing freely, sooty at maturity, 100–250 μ diam.

Spores rather long fusoid, straight or somewhat inequilateral, 5-celled 21–25 × 5.5–6.6 μ; the three central cells guttulate, 15–16 μ, the two upper colored darker than the lower; exterior cells hyaline, basal long conic, apical long cylindric with 3, rarely 4 setae, 22–30 μ; pedicel 4–14 μ (Fig. 4, *12*).

On dead leaves of *Vaccinium macrocarpon*, Parkdale, New Jersey, Sept. 1901, C. L. Shear.

While this species may have some resemblance to Desmazières' illustration of *P. guepini* (Ann. Sci. Nat. Bot. II. **3**: 182, pl. 4, fig. 1–3, 1848),

it differs decidedly from the type in Desmazières Pl. Crypt. de France **22**: *1084*, and from other exsiccati specimens. This fungus is sufficiently characteristic to deserve recognition as a distinct species.

According to Shear (72, pp. 38–39) *P. vaccinii* is generally distributed in cranberry-growing regions, specimens having been obtained from West Virginia, New Jersey, Wisconsin, Massachusetts, and New York. Leaves and fruit are affected, but the fungus does not attack fruit with sufficient frequency to cause much loss. It has been found on leaves which show no external signs of disease. N. E. Stevens (77) considered this fungus as one of the many organisms causing decay of cranberries. He recovered it from rotted green berries and from healthy green berries as early as the middle of July.

4. PESTALOTIA CESATII Rabenhorst, Fungi Europaei, *1932*. 1875.

Fruiting bodies largely hypophyllous, scattered, at first subepidermal, then erumpent, rupturing the epidermis usually in a stellate manner and exposing the black pustules, 96–160 μ wide.

Conidia 5-celled, broad fusiform, erect, rarely curved, slightly constricted at the septa, 24–26.5 μ; 3 median cells olivaceous, equally colored, 16–19 μ × 8.5–9.5 μ; end cells hyaline, the basal short, conic, or obtuse, supported by a coarse pedicel, 4–12 μ; apical cell short, conic, bearing a crest of usually 5 short, erect or acutely divergent setae, 7–19 μ, frequently knobbed at the extremities and somewhat unequal in length (Fig. 3, *6*).

On moist leaves of *Podocarpus latifolia* in Botanic Gardens, Naples, Cesati, in Rab., F. Eur. *1932*.

5. PESTALOTIA FUNEREA Desm., Ann. Sci. Nat. Bot. II. *19*: 335–336. 1843; Sacc. Syll. Fung. **3**: 791. 1884; Klebahn, Myc. Centralbl. **4**: 5. 1914.

P. conigena Lév., Ann. Sci. Nat. Bot. III. **5**: 285. 1846; Sacc. Syll. Fung. **3**: 792. 1884; Klebahn, Myc. Centralbl. **4**: 6. 1914.

P. abietina Roumeguère, Fung. Sel. Gall. Ex. *735*. nomen nudum.

Pustules amphigenous, 100–250 μ in diameter, usually scattered, sometimes confluent, black, subepidermal, later erumpent and surrounded by the torn epidermis, on maturity liberating the sooty contents.

Spores clavate or elliptic-fusoid, 21–27 × 7–9 μ, equilateral, 5-celled; median colored cells guttalate, umber, the lower sometimes brighter, 15–17.5 μ, only slightly constricted at the septa, tapering at the lower end; exterior cells hyaline, the apical cell usually small, somewhat inconspicuous, bearing a crest of 3, 4, or 5 but rarely 6 setae, usually simple and erect, 5–20 μ; basal hyaline cell usually long conic supported on an erect pedicel, 2–7 μ (Fig. 3, *8* and *9*).

On leaves, bark, and cones of *Thuja orientalis, T. occidentalis, Retinospora squarrosa, Chamaecyparis spp., Abies lanceolata, Cupressus spp.* and related species. The type fungus was described on dead leaves of *Thuja.* The original material was not accessible but study of specimens on coniferous plants, whose characters agree with Desmazières' type description, leaves no doubt of their identification. The usual presence of 4–5 erect setae, broad fusoid spores, dark olivaceous colored median cells, and the small apical hyaline cell are particularly characteristic of this fungus.

Specimens examined; on *Abies lanceolata* (leaves); Roumèguere, Fung. Sel. Gall. Ex. *735.* Main et Loire, Angers, France. Guepin, sub *P. abietina* n. sp. (*P. guepini* Desm. var. *abietina* Guepin Mss.)

On *Chamaecyparis lawsoniana* (leaves and twigs); Sydow, Myc. Germ. *839.* Tamsel, Brandenburg, Germany, Apr. 1, 1909. P. Vogel; Kabat and Bubak, Fung. Imp. Ex. *739,* Febr. 7, 1911.

On *Cupressus sp.,* Rab. Herb. Myc. Ed. Nov. 1: 63, Brixia, Italy, Cesati sub. *P. funerea* Desm. var. *heterospora*; on *C. macrocarpus* (bark), Ellis, No. Amer. Fung. *634,* California, Harkness.

On *Retinospora squarrosa* (leaves); Sydow, Mycoth. March. *4440,* Bellevue Garten, Berlin, Germany, Oct. 1895, P. Sydow.

On *Thuja orientalis elegantissima* (leaves); Shiloh, N. J. Sept. 9, 1927, White; on *T. occidentalis* (cones), Ravenel Fung. Amer. Ex. *700,* Aiken, So. Carolina; Herb. N. Y. State Mus., Oneida, N. Y., June 8, 1923, H. D. House; Ellis No. Amer. Fung. *527,* Sept. 1880, sub *P. conigena* Lév.; (leaves) Dearness, *3325,* and Barth. Fung. Col. *3535,* London, Canada, Aug. 1911, J. Dearness.

Much confusion exists regarding the identity of this fungus. Specimens on a wide range of host plants have been incorrectly named. The fungus is restricted to plants of the Pinaceae. Both Saccardo and Rabenhorst have listed several hosts in many families and Doyer (25) consequently has stated that the fungus is omnivorous.

P. funerea Desm. and *P. conigena* Lév. are identical. The type of the latter occurs on cones of *Thuja occidentalis* and *Pinus sylvestris.* Léveillé's description is extremely indefinite. All statements of size and color are lacking and the number of septa is either 3 or 4. On the other hand, such characters as "sporis elongatis in stipitem attenuatis—filis 4 pellucidis" and the obtuse apex are suggestive of the similarity of Léveillé's fungus. The early conceptions of *P. conigena* were contradictory. Some mycologists regarded it as a 4-celled spore form, others as having 5 cells. Saccardo (Syll. Fung. **3**: 792. 1884) reported 4 septa with 3 dark median cells, and Klebahn (Myc. Centralbl. **4**: 6. 1914), Voglino (87), and Tubeuf (82, pp. 40–51) adhere to this view. Tubeuf and Voglino have established that the

4-celled spore form in Rabenhorst Fung. Eur. *2462* is not *P. conigena* Lév. and both have described it as a new species, Voglino as *P. abietina*, and Tubeuf as *P. conorum-piceae*. Both of these names are synonymous with *P. stevensonii* Peck. Gleisberg (34) regarded both *P. conigena* Lév. and *P. foedans* Sacc. & Ell. synonymous with *P. funerea*. My study shows that *P. foedans* is a distinct species.

Doyer's interpretation of *P. funerea* is confusing. Without knowledge of the type, and basing her conception of this species on descriptions by Saccardo and Klebahn, she claimed to have isolated this fungus from *Rhododendron* and various conifers. On the basis of descriptions of *P. macrotricha* Kleb., *P. conigena* Lév. and *P. foedans* Sacc. & Ell. furnished by these workers, she assigned forms isolated from *Taxus, Retinospora*, and *Biota* to *P. macrotricha* and those from *Thuja, Chamaecyparis, Juniperus*, and *Pinus* to *P. funerea*. According to my studies *P. macrotricha* is limited to *Rhododendron* and shows no similarity with forms on conifers.

The validity of varieties within this species has been doubted by Gleisberg (34) and Doyer (25). My own studies indicate that the assumed relationship of the numerous described varieties of *P. funerea* is incorrect. It is also evident that this species is not of wide host range. Disposition of the described varieties of the species is reserved for a future paper.

Differences of opinion exist concerning the biology of *P. funerea*. There is some evidence in favor of its parasitic habit. Böhm (10) reported this species as causing the blight of distal shoots of *Thuja menziesii*. He was successful in obtaining infection with spores applied to wounds on living twigs of *Thuja menziesii, Chamaecyparis lawsoniana*, and *Pseudostuga douglasii*, but after five months no spores had developed. The extensive dying of junipers at Eberswalde which Böhm attributed to *P. funerea* is claimed by Danckelmann (21) to be winter killing from temperatures of $-30°$ to $-32°$ C. in the winter of 1892–1893. Gleisberg (34) inoculated living parts of *Thuja occidentalis* with spores of *P. funerea* obtained from bark of the same host but without success, and he concluded that *P. funerea* was a saprophyte which developed only after a change in the chemical and physical nature of the bark.

Spaulding (74) reported a serious needle blight of *Pinus ponderosa* and *P. divaricata* on two-year-old seedlings from which he recovered *P. funerea*. With this inoculum he succeeded in infecting healthy seedlings of *Pinus ponderosa*. A similar disease has been reported from South Africa by Fisher (29) on *Pinus pinaster, P. canariensis, P. longifolia, P. insignis, P. massoniana, Cupressus lusitanica, Chamaecyparis lawsoniana*, and *Casuarina leptoclada*. The organism was identified as *P. funerea* but no proof of its pathogenicity was established. Hartley, Merrill, and Rhoads (37) re-

ported *P. funerea* very common on dead coniferous needles throughout the United States. With cultures obtained from jack and Rocky Mountain yellow pines, inoculations of white pine were without result. Attempts to infect green shoots of *Thuja occidentalis*, some of which had been injured by puncturing or heating, with spores from the same plant failed.

In an earlier paper Hartley (36, pp. 15–18) reported having isolated 4- and 5-celled spore forms of *Pestalotia* from damping-off coniferous seedlings, but that cultures were not sufficiently frequent to indicate parasitism. Smith (73) found *P. funerea* associated with withered distal portions of shoots of *Biota orientalis* in England, and reported a similar phenomenon on species of *Thuja, Cupressus, Chamaecyparis,* and *Juniperus.* He assumed the fungus to be parasitic because Tubeuf (82, pp. 40–51) earlier regarded *P. hartigii* as the cause of the "Einschnürungskrankheit" of forest tree seedlings in Germany. Wenner (89) isolated *P. funerea* from bark and needles of *Pinus strobus* and his inoculation experiments proved conclusively that this species is parasitic under certain conditions, the most important of which is the presence of moisture. The fungus was shown to be capable of attacking leaves and stems of white pine, Norway spruce, and hemlock. A needle blight of white pine very prevalent in Connecticut in 1907 was attributed by Clinton (16, 17) to adverse weather conditions. Studhalter (80) infected artificially the needles of *Pinus radiata* apparently with *P. funerea.* Graves (35) found a *Pestalotia* on needles of withered shoots of Norway spruce, and the manner of the appearance of the disease suggested a parasitic relationship.

Doyer (25) inoculated seedlings of *Pinus sylvestris* and *Picea excelsa* in wounds, in spots injured by heat, and elsewhere with *P. funerea* from *Thuja* and *Chamaecyparis*; with a form from *Taxus, Biota,* and *Retinospora* which she named *P. macrotricha*; with a form from *Thuja* which she named *P. versicolor* Speg.; and with Fischer's strain of *P. hartigii.* But in no case was infection obtained. Negative results were also obtained on the same plants with Brown's strain of *P. scirrofaciens* Brown, *P. monochaetioides* Doyer, *P. virgatula* Kleb., and *P. theae* Saw. Further inoculations with the same forms of a number of conifers under various conditions and at different times of the year failed. The existing differences of opinion of the biology of *P. funerea* which this review indicates justify further study.

6. PESTALOTIA FOEDANS Sacc. & Ellis, Michelia **2**: 575. 1882; Sacc. Syll. Fung. **3**: 792. 1884.

Pustules densely gregarious, elongate and hysteriform, innate erumpent, coalescing freely, rupturing bark in a longitudinal manner, 200–450 × 90–270 μ, twice or more as long as wide, contents blackening the matrix.

Conidia fusoid, 5-celled, 19–24 × 5.5–6.5 μ, equilateral, erect, only slightly constricted at the septa; the dark interior cells 14–16.5 μ, guttulate, the upper two umber, the lower olivaceous; the basal cell prominent, rather broad-conic, the apical small, acute, bearing at its apex a crest of 3, rarely 2 or 4, slender, divergent setae, 10–17 μ; pedicel short, straight, 4–8 μ (Fig. 3, *5*).

On decaying bark of *Chamaecyparis thyoides*. Masses of acervuli and spores cover the affected surfaces.

Specimens examined: Ellis, No. Amer. Fung. *954*, Newfield, N. J., Oct. 1880, Ellis and Harkness, and similar specimens in E. and E., Fung. Col. *81*, and Rab., Fung. Eur. Ex. *2997*.

7. **Pestalotia glandicola** (Cast.) Guba n. comb.

Robillarda glandicola Castagne, Cat. Pl. Marseille 205, Pl. IV. 1845.

Pestalotia castagnei Desm., Ann. Sci. Nat. Bot. III. **6**: 64. 1846; Desm. Pl. Crypt. de France **30**: 1490. 1846; Sacc. Syll. Fung. **3**: 793. 1884.

Pustules sub-epidermal, erumpent on maturity, coalescing freely, forming dense black cushions, globose or lenticular, tearing covering irregularly, 160–480 × 160–320 μ.

Spores 5-celled, fusiform, somewhat curved, 22.5–25 μ; median cells dark, stricted at septa dividing the two lower colored cells; end cells rather large, the upper two fuliginous, the lower olivaceous, 15.5–18 × 8.4–9.5 μ, conprominent, the apical conic to cylindric, the basal conic, tapering; setae 3, divergent, 9–19.2 μ; pedicel 2.4–7.2 μ, erect (Fig. 2, *1*).

On dried fallen acorns of *Quercus ilex* from France, in Desm. Pl. Crypt. de France **30**: *1490*. 1846 and Curtis Herbarium at Harvard. The pustules are sub-epidermal. The black contents issue in thick coarse threads and on being moistened spread out around the perforations. Three hyaline, filiform setae shorter than the spores surmount the apical cell. The setae often spread out at right angles and rarely lie down over the spore. Castagne originally described this species as having 3-celled spores, with a central colored cell and hyaline exterior cells. Setae were not mentioned. Type specimens distributed by Castagne bear only spores of *Pestalotia*.

8. PESTALOTIA SPHAERELLOIDES Ellis & Langl., n. sp., Sacc. Syll. Fung. **13**: 268. 1898. nomen nudum.

Acervuli hypophyllous, subglobose, scattered but gregarious in places, like pycnidia, raised, erumpent, circinate, surrounded by the torn epidermis, 100–150 μ wide.

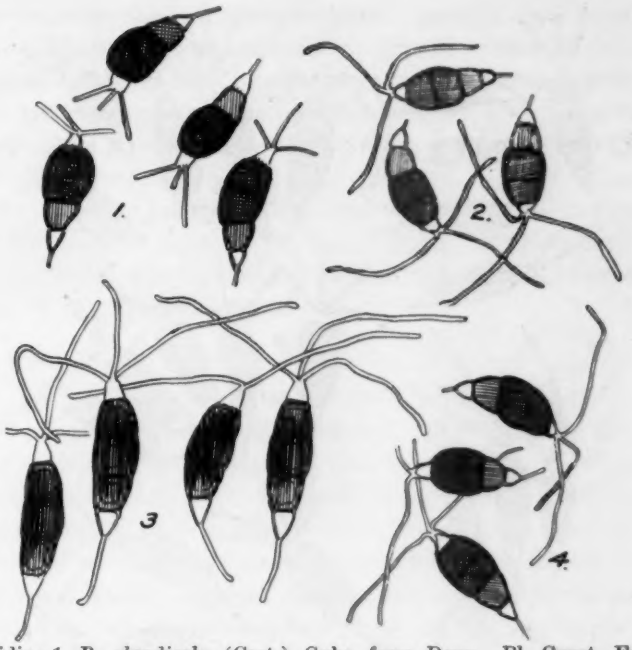

FIG. 2. Conidia: *1, P. glandicola* (Cast.) Guba from Desm., Pl. Crypt. Fr. Fasc. 30, *1490*, on acorns of *Quercus ilex*, sub *P. castagnei* Desm.; *2, P. scirpina* E. and M. from E. and E., No. Amer. Fung. 2d. Ser. *2181*, on dead culms of *Scirpus olneyi*; *3, P. macrotricha* Klebahn from E. and E., Fung. Col. *370a*, on *Rhododendron maximum*, sub *P. guepini* Desm.; *4, P. sphaerelloides* Ellis. and Langl. from E. and E., No. Amer. Fung. *2387*, on leaves of *Carya aquatica*. × 750.

Spores 5-celled, erect, equilateral, broad-clavate, constricted at septum dividing the two lower colored cells, 19.5–25.5 μ; median cells dark colored. the upper 2 fuliginous, the lower olivaceous, 15–17 × 8.5–9.5 μ; end cells hyaline, conspicuous, elongate, the apical cell bearing 2 or usually 3 long, widely divergent setae, 19–30 μ; pedicel 4–7 μ (Fig. 2, *4*).

On leaves of *Carya aquatica*, Bayou Chêne, Louisiana, Oct. 1888, A. B. Langlois in E. & E., No. Amer. Fung. *2387*. Distinct pycnidium-like pustules are scattered generally over the lower surface of the leaves.

9. PESTALOTIA CLIFTONIAE Tracy & Earle, Bul. Torrey Bot. Club **22**: 178. 1895; Sacc. Syll. Fung. **14**: 1027. 1899.

Acervuli epiphyllous, punctiform, scattered, erumpent, circular, 120–200 μ wide, seated on orbicular or circular pale or brown spots not exceeding 1 cm. in diameter with definite narrow purple margins.

Conidia obovate, usually curved, unequilateral, 5-celled, constricted at the septum dividing the 2 lower colored cells, 19–24 μ, septa often oblique;

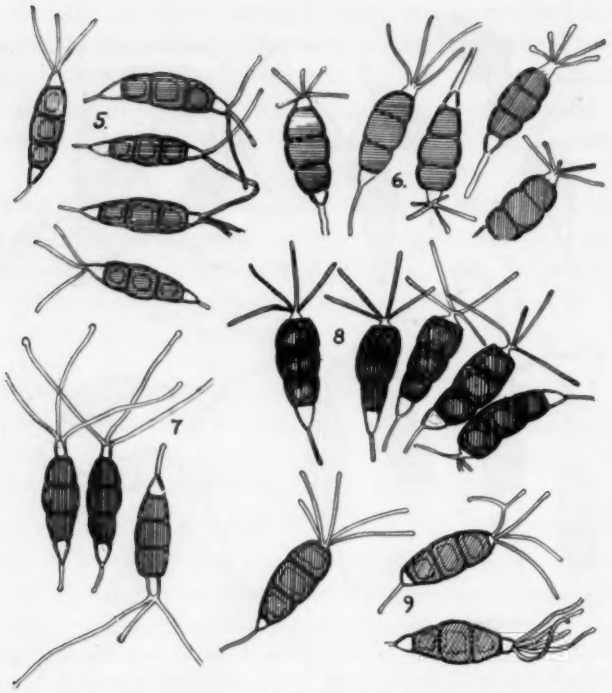

FIG. 3. Conidia: *5, P. foedans* Sacc. & Ellis from Ellis, No. Amer. Fung. *954*, on decaying cedar bark, Newfield, N. J. Oct., 1880, Ellis and Harkness; *6, P. cesatii* Rab. from (Rab.) Fung. Eur. *1932*, on leaves of *Podocarpus latifolia*, Bot. Gardens, Naples. Cesati; *7, P. theae* Sawada from Sydow Fung. Exot. Ex. *146*, on leaves of *Camellia theae*, sub *P. palmarum; 8, P. funerea* Desm. from Ellis, No. Amer. Fung. *527*, on fallen cones of *Thuja occidentalis*, sub *P. conigena; 9, P. funerea* Desm. from Roumeguère Fung. Sel. Gall. Ex. *735*, on *Abies lanceolata*, Angers, France, Guepin, sub *P. abietina* Roumeguère. ×750.

3 median cells dark colored of which the upper 2 are fuliginous and opaque, the lower olivaceous, 14.4 × 8–9 μ; end cells hyaline, the apical cell short, conic, bearing a crest of 3 widely divergent setae, 16–25.6 μ, the basal cell short, obtuse, abruptly contracted into a short stipe, not exceeding 5 μ.

On living leaves of *Cliftonia ligustrina*, Ocean Springs, Mississippi, Nov. 1893, associated with ash or pale brown leaf spots, Herb. of F. S. Earle, Myc. Coll., U. S. Department of Agriculture.

10. PESTALOTIA SCIRPINA Ellis & Martin, Amer. Nat. **19**: 76. 1885.

Pustules lenticular to long hysteriform, scattered and distinct, subcuticular, later rupturing the cuticle longitudinally, contents at length oozing out and staining the matrix, 140–1000 × 110–140 μ.

Conidia fusiform, 5-celled, erect or usually curved, constricted at septa dividing the two lower colored cells, 20–24 × 6.5–8.5 μ; median cells dark colored, the upper two fuliginous, opaque, the lower olivaceous, 15–17 μ; apical hyaline cell conic, elongate, sometimes tapering, basal hyaline cell broad, conic, supported on an erect pedicel, 3.5–10 μ; crest mostly of 3 setae, sometimes 2, curved, divergent, 18–27 μ (Fig. 2, *2*).

On culms of *Scirpus maritimus,* Shores of Chesapeake Bay, Md., July, 1884, J. T. Rothrock in E. & E. No. Amer. Fung. *1626*; on dead culms of *Scirpus olneyi,* Point à la Hache, La., March, 1887, A. B. Langlois in E. & E. No. Amer. Fung. 2nd Ser., *2181.*

11. PESTALOTIA CURTA Sacc. Bul. Soc. Bot. Ital. **1904**; 209, fig. 5. 1904; Sacc. Syll. Fung. **18**: 480. 1906.

Acervuli usually concentrically disposed, largely epiphyllous, prominent, breaking through the epidermis in elongate fissures, 160–220 × 80–230 μ, issuing forth in dense black masses, irregularly scattered and sometimes confluent, seated on sub-circular, dark brown spots or areas of different shape usually marked by concentric zones above and lacking them below.

Conidia broad-clavate, 5-celled, usually curved and unequilateral, 20–24 μ long; median cells 14–16 × 8.5–10.5 μ, colored, the two upper umber or fuliginous, the lower olivaceous, constricted at the septum dividing the two lower cells; basal hyaline cell broad conic and small, superior cell narrow conoid or tapering and sometimes hidden, bearing 3 or rarely 2 or 4 setae which are sometimes branched, 16–30 μ; pedicel erect, 2–5 μ (Fig. 4, *11*).

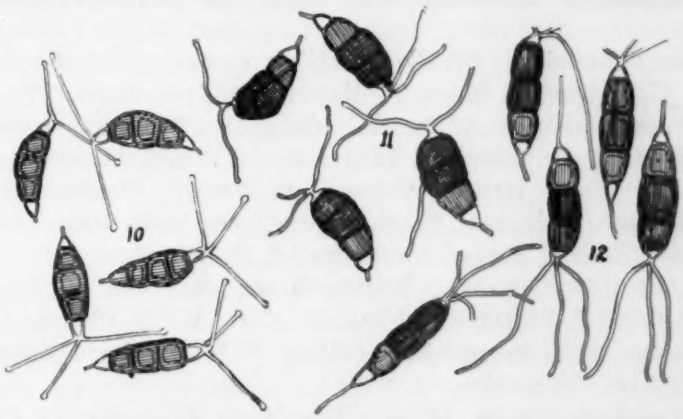

FIG. 4. Conidia: *10, P. palmarum* Cke. from Sydow Fung. Exot. Ex. *289,* on leaves of *Cocos nucifera,* Phillippines; *11, P. curta* Sacc. from D. Sacc. Myc. Ital. *1570,* on leaves of *Ceratonia siliqua; 12, P. vaccinii* (Shear) Guba from Myc. Coll., U. S. D. A., on leaves of *Vaccinium macrocarpon,* Parkdale, N. J. Sept., 1901, Shear, sub *P. guepini* Desm. var. *vaccinii* Shear. ×750.

On wilting leaves of *Ceratonia siliqua*. Associated with reddish or brown sub-circular spots or areas of different shape which sometimes coalesce.

Study is based on type specimens of *P. curta* Sacc. in D. Sacc. Myc. Ital. *1570,* Botanic Garden, Padua, on leaves of *Ceratonia siliqua,* April, 1904.

12. PESTALOTIA PALMARUM Cke. Grevillea **4**: 115. 1875, and **5**: 102. 1876. Pl. 86, fig. 3: Sacc. Syll. Fung. **3**: 796. 1884; Klebahn, Myc. Centralbl. **4**: 9. 1914.

P. phoenicis Vize, Grevillea **5**: 14. 1876; Sacc. Syll. Fung. **3**: 796. 1884; Klebahn, Myc. Centralbl. **4**: 11. 1914.

P. brevipes Prillieux and Delacroix, Bul. Soc. Myc. France **10**: 84–85. 1894; Sacc. Syll. Fung. **11**: 788. 1895; not Cke.

P. palmicola Sacc. and Syd. in Sacc. Syll. Fung. **14**: 1030. 1899.

Pustules black, sparse, sub-epidermal, later erumpent, amphigenous, 125–425 × 100–260 μ, circular or broad elliptic from above, sometimes coalescing, seated on spots or present generally on the dead areas.

Spores fusiform, straight or curved, 5-celled, 16.5–21 × 4.8–6.5 μ; interior cells olivaceous, the two upper occasionally darker, somewhat constricted at septa, 12–14 μ; exterior cells conical; setae 2 or 3, usually straight and spreading outward, 8.5–20 μ, often knobbed; pedicel short, 2–6.5 μ (Fig. 4, *10*).

On leaves of *Cocos nucifera* and other species of palm, associated with pale dead areas of various shapes surrounded by definite, reddish brown borders.

Specimens examined: On *Cocos nucifera* (sprout); Kew Herb., India, Jan. 27, 1870; (leaves) Baker, F. Malayana *65,* Los Banos, Philippines; Hume, Fungi of Florida *53,* Sanibel Island, Dec. 28, 1899; Roumeguere, Fung. Sel. Gall. Ex. *5166* and Thüm., Myc. U. *1676,* Demerara, British Guiana, M. C. Cooke, Herb. J. A. Stevenson *2220,* Rio Piedras, Porto Rico, 1914, Stevenson; Philippine Fungi *23,* Los Banos, O. H. Reinking; Sydow Fung. Exot. Ex. *289,* Taytay, Philippines, E. O. Merrill.

On *Howea foresteriana* (=Kentia foresteriana) and *H. belmoreana* (leaves); Office F. Pl. Quar. and Insp. U. S. D. A. *714,* Philadelphia, Pa., in greenhouse, J. T. Rogers; Myc. Coll. U. S. D. A., Florida, sub *P. discoidea* Ellis, nomen nudum.

On *Phoenix dactylifera* (leaves); Vize. Micro. Fung. Exot. *7,* Col. Hobson, sub *P. phoenicis* Vize; Kew Herb., East Indies, Col. Hobson, 1876, sub *P. phoenicis* Vize.

This species was described by Cooke, first on a dead sprout of germinating coconut from Bengal, India (spores 0.015 mm. long), and later on dead

leaves of the same host from Demerara, British Guiana (colored part of spores 0.045 mm. long). Both are *P. palmarum* but the dimensions given in each case are incorrect. The spores of the type on sprout of coconut from India lack the knobbed setae; otherwise the fungus is similar to the specimens later described from Demerara, British Guiana. Klebahn (43) considered *P. phoenicis* Vize a distinct species, but type material at Kew collected by Col. Hobson shows sufficient agreement with *P. palmarum* Cke. to regard Vize's name as a synonym. *P. brevipes* Prillieux and Delacroix is synonymous with *P. palmarum* Cke. Saccardo and Sydow (Sacc. Syll. Fung. **14**: 1030. 1899) renamed *P. brevipes* (Sacc. Syll. Fung. **11**: 788. 1895) to *P. palmicola* Sacc. and Syd. because there already existed a *P. brevipes* Cke. on *Aralia spinosa*. Both names, therefore, become synonyms. *P. discoidea* Ellis, on leaves of *Howea forestieriana*, is the same as *P. palmarum* Cke. The name and description of this fungus were never published, but specimens of the type accompanied by a description in Ellis' hand writing are deposited in Mycological Collections, United States Department of Agriculture.

Confusion has resulted from Cooke's descriptions of *P. palmarum*. The incorrect application of this name to other species of *Pestalotia* on a wide variety of plants has appeared extensively in mycological and pathological literature. Bernard (3, and 5, pp. 1–16) regarded the *Pestalotia* on tea and *P. palmarum* identical but it also appeared to Bernard that *P. guepini* and *P. palmarum* were distinct species and that the former was not the cause of the gray blight. Doyer (25) contended that *P. palmarum* and *P. guepini* were identical; but Sawada (69) regarded the tea fungus as a distinct species which he named *P. theae*; and Butler (13), who studied type material of these three species, confirmed their distinctiveness. Archer (1) confirmed the individuality of *P. palmarum* and *P. guepini* by their comparison in artificial culture; and Bertus (8, 9), by morphologic and cultural studies of *P. theae* and *P. palmarum*, reported these species distinct. My studies recognize the individuality of these species.

There has been much doubt concerning the biology of *P. palmarum*. Bernard (4) obtained infection on coconut palm by placing a cluster of diseased leaves in a healthy isolated tree; after two months, disease appeared. An uninoculated tree nearby remained healthy. He also found that a single conidium was capable of penetrating the leaf at several points, producing several spots close to one another. He reported that infection is stomatal, which fact has been confirmed by Stockdale (78). Stockdale found that the fungus was also capable of infecting the leaves through wounds and that it was frequently accompanied by *Diplodia epicocos*, a saprophyte which completes destruction begun by *P. palmarum*. Leefmans

(51) found that in Java the injury caused by the adults and larvae of the two-colored coconut leaf beetle, *Bronthispa froggatti*, was increased by *P. palmarum*, which penetrated the young leaves through injuries made by this insect. Bertus (9) found that *P. palmarum* is able to infect coconut leaves through wounds but not healthy tissue.

Doyer (25) failed to infect *Kentia foresteriana* with a form from *Cocos*, with a form from *Rhododendron* incorrectly named *P. guepini*, with Shear's strain of *P. guepini* Desm. var. *vaccinii* Shear [= P. vaccinii (Shear) Guba], and with a form from coconut incorrectly named *P. theae*. She concluded that the palm disease is of non-parasitic nature. Petch (60, p. 83; 61, pp. 226 and 262; and 62, pp. 163–164), Rutgers and Arens (66), and Rutgers and Dammerman (67) attributed the dry bark-girdle disease of rubber seedlings to *P. palmarum* Cke., but Steinmann (76) reported this disease due to sunburn.

The leaf spot of palms is prevalent in the tropics according to Morstatt (55, pp. 234–236), but its occurrence, according to Stockdale (78, 79) in the West Indies, Bernard (4, and 6, pp. 38–43) in Java, Knowles (44, pp. 26–27) in the Fiji Islands, and others is restricted to old or weak trees and unfavorable conditions for growth. Kowalski (45, pp. 56–58) reported that the disease occasioned considerable loss of young palms in the New Hebrides. Bernard (4) reported one-year-old coconut plants most susceptible in Java, and that the fungus caused great damage to coconut plantations. In the tropics in general this leaf disease is not of much concern and the primary factors responsible for damage have in most all cases been overlooked and wrongly attributed to *P. palmarum*. Prillieux and Delacroix (65) reported damage to species of *Kentia*, *Chamaerops*, and especially *Corypha australis* by the palm fungus in greenhouses at Versailles, France. Bernard (6, pp. 38–43) reported the fungus on *Oredoxa*, *Elais guinensis*, *Maniltoa gemmipara*, *Palaquium*, *Myrmecodia echinata*, *Thea*, and *Hevea brasiliensis*. A leaf disease of *Hevea* has also been attributed to *P. palmarum* by Bernard (7, pp. 43–44), Petch (60, 61, 62), Weir (88, p. 88), and others, but my studies indicate that the palm fungus is limited to members of the palm family.

13. PESTALOTIA THEAE Sawada Spec. Rept. Agr. Exp. Sta., Taiwan, Formosa, **11**: 113, pl. 4, figs. 7–9, T. 4, 1915 [Japanese; tr. Tanaka, Mycologia 9: 171, 1917].

Brownish spots coalescing and forming irregularly margined patches of variable extent especially common along leaf margins. Spots 1 cm. or more in diameter marked by concentric rings of alternate lighter and deeper shades.

Pustules punctate, chiefly epiphyllous, often arranged in concentric circles or scattered without order, 88–176 μ diam., sub-epidermal, on rupturing forming black crusts.

Spores long-fusiform, erect, 4-septate, slightly constricted at the septa, 23–30 × 7–8 μ; 3 middle cells 16.5–19 μ, dark olivaceous; end cells hyaline, 4–6 μ long, basal cell conic, apical cell cylindric with 2–4, commonly 3, hyaline, filiform appendages, 25–48 μ, the free ends usually knobbed; stalk filiform, rigid or curved to one side, 5–9 μ, sometimes knobbed (Fig. 3, *7*).

Fungus occurs on living leaves of tea causing disease known as gray blight. Large pustules appear on concentric zones but on the lower leaf surface the zones are not always distinct and the pustules are usually few. The fungus occurs generally in tea-growing regions.

Specimens examined: Sydow Fung. Exot. Ex. *146* Peradeniya, Ceylon, on leaves of *Camellia thea* (*Thea sinensis*) Nov. 1912, Petch, sub *P. palmarum* Cke.; Ceylon, May, 1927; Buitenzorg, Java, May, 1927.

Like *P. palmarum* the identity of the tea fungus was not known until Butler (13), Sawada (69), and Bertus (8, 9) recognized it as a distinct species, *P. theae* Sawada. Previously the fungus was incorrectly designated *P. guepini* Desm. and regarded of cosmopolitan host range.

P. theae attacks leaf blades, petioles, and stems of tea. The fungus grows from the blades through the petioles into perennial parts of the bush and causes cankers. Carruthers (14, pp. 4–7, and 15, pp. 5–6) and Petch (58, 59, and 63, pp. 27–30, 106–107, 191, 211) reported destruction of shoots following infection at the cut ends of stems left in gathering the tea, leaving a "crow's foot" structure of half a dozen dead twigs. Tunstall and Bose (86) successfully infected the cut shoots of tea with the gray-blight fungus. They reported that it frequently attacks margins of young immature leaves after a period of hot, dry weather causing rim blight, and that the attacks of red spider also make leaves susceptible to infection.

Carruthers (15) observed in Ceylon that gray blight appears after bushes are allowed to go 18 months without pruning. According to Tunstall (85, pp. 77–80) attacks of gray blight are an indication of unsatisfactory soil conditions. The fungus does not attack vigorous healthy leaves. Its attacks are confined to old damaged bushes or to those previously weakened by some other cause. Bertus (9) found that *P. theae* was able to infect tea and coconut leaves through wounds but not healthy leaves, while Doyer (25) failed to obtain infection of tea with a *Pestalotia* from *Cocos*, incorrectly designated *P. theae*, and with a *Pestalotia* from *Cocos* incorrectly designated *P. guepini*. Doyer regarded forms from *Cocos nucifera* and *Altingia* as *P. theae* Sawada because of the knobbed setae. She was apparently unaware of the fact that the setae of *P. palmarum* Cke. also have

knobbed extremities. Since *P. theae* is restricted to tea, inoculating tea with forms from palm, as Doyer has done, might be expected to give negative results. Speshnev (75) observed at Chakva, near Batum, that *Thea sinensis* was particularly susceptible and that the native species *Thea assamica* suffered much less damage. Other accounts of gray blight are made by Delacroix (22, p. 71), Delacroix and Maublanc (23, pp. 415–419), and Bernard (3, and 5, pp. 1–16).

14. PESTALOTIA MACROTRICHA Klebahn, Myc. Centralbl. **4**: 6. 1914.

Acervuli amphigenous, usually epiphyllous, subglobose or lenticular, black, punctiform, 140–280 μ wide, subepidermal, then erumpent, circular, surrounded by the torn epidermis, often densely gregarious and confluent, sooty on drying and spreading over the leaf surface.

Spores 25–31 × 7.4–9.4 μ, erect or sometimes curved, long fusoid, tapering to the base, 5-celled; 3 median cells olivaceous, long cylindric, equally colored or the upper two slightly darker than the lower, 18–23 μ, only slightly constricted at the septa; exterior cells prominent, the upper long cylindric with a crest of 3 or sometimes 2 or 4 coarse, long, divergent setae, 17–40 μ; the basal cell long conic with a long erect pedicel, 8–17 μ (Fig. 2, *3*).

On stems and leaves of *Rhododendron* and *Azalea* associated with definite dark or pale brown spots and areas commonly confined to the tips and along the margins of the leaves, confluent frequently and causing defoliation. The fungus appears on stems as black, raised pustules scattered without order.

Specimens examined: E. & E., Fung. Col. *370a* (type) on leaves of *Rhododendron maximum*, Nuttalburg, W. Va. Nuttal, sub *P. guepini* Desm.; on Rhododendron hybrids, Boskoop, Holland. Washington Insp. House, May 26, 1920, J. A. Stevenson; on *Rhododendron maximum*, Holland, Herb. J. A. Stevenson *1359*, Apr. 9, 1921; on *Rhododendron sp.* Westchester, Pa., 12–14–1914, F. Windle; on *Rhododendron ponticum*, Dedemsvaart, Holland, Washington Insp. House, 4–4–23, A. J. Bruman; brown fallen leaves of *Rhododendron* from Belgium, Washington Insp. House, 10–28–15, J. T. Rogers, Pl. D. S. *471*, sub *P. guepini* Desm.; on *Rhododendron sp.* Naarden, Holland, Washington Insp. House, Apr. 10, 1921, A. J. Bruman and H. F. Bain.

The long fusoid spores, olivaceous colored interior cells, the upper two of which at maturity are sometimes darker, long hyaline exterior cells, long setae and pedicel are characteristic of this species. The black raised fruiting pustules on leaves and stems are also typical of the fungus. Doyer's strains from *Taxus*, *Retinospora*, and *Biota* which she named *P. macrotricha*

Kleb. are not this fungus. No species occurring on conifers show any agreement with the one on *Rhododendron*.

The stem and leaf blight of *Rhododendron* or *Azalea* are commonly encountered in shipments of stock from Holland and France, and the disease causes considerable damage to rhododendrons, especially in nurseries. Clinton (18) reported the disease on imported rhododendrons in Connecticut, and Flachs (30) described the leaf blight on *Azalea* in Germany imported from Belgium. Schwarze (71, p. 112) reported *Pestalotia* on sunburnt leaves of *Rhododendron* in New Jersey which he named *P. guepini*, and I have found *Monochaetia* and other fungi on similarly affected foliage.

Schmitz (70) reported that the fungus is often found on leaves that have suffered from activities of a small aphid. By the usual methods of artificial inoculation he obtained positive evidence that this form is parasitic in nature. Tengwall (81, p. 58) also obtained infection. Doyer (25) inoculated rhododendron with a strain from *Cocos* incorrectly determined as *P. guepini* Desm., with Shear's *P. guepini* Desm. var. *vaccinii* Shear [=*P. vaccinii* (Shear) Guba] from cranberry, with strains from *Juniper* and *Rhododendron* named *P. funerea*, and a strain from *Retinospora* incorrectly named *P. macrotricha*. She obtained the rhododendron *Pestalotia* from the same plant (*R. ponticum*) on which Tengwall originally found his strain and with it inoculated the Pink Pearl rhododendron and *R. ponticum*. Leaves of rhododendron were also inoculated with two strains isolated from *Rhododendron* incorrectly named *P. guepini*, *P. funerea* from *Thuja*, a strain from *Taxus* incorrectly named *P. macrotricha*, and a strain from *Altingia* incorrectly named *P. theae*. Inoculations in every case were unsuccessful, and Doyer concluded that the form on rhododendron is not a parasite.

15. **Pestalotia rhododendri** (D. Sacc.) Guba, n. sp.
P. versicolor Speg. var. *rhododendri*, D. Sacc. Myc. Ital. *978*. 1902. nomen nudum.

Pustules black, epiphyllous, densely gregarious or scattered, often confluent, erumpent on maturity, tearing the epidermis irregularly, the black contents spreading over the matrix, 140–400 μ in diameter, seated without order on dried brown areas of living leaves.

Spores 5-celled, broad fusiform, erect or slightly curved, not constricted at septa, 21–27 μ; median cells stout and short, guttulate, the two upper fuliginous, the lower olivaceous, 16.5–18.7 × 8–10 μ; end cells hyaline, prominent, apical cell broad conic or short cylindric, basal cell broad conic, abruptly contracted into a short straight pedicel, 4–6.5 μ; setae 2 or usually 3, rarely branched, curved and widely divergent, 17–35 μ (Fig. 6, *22*).

On blighted or living leaves of *Rhododendron* associated with spots or dead areas usually along the margins and at the apex.

Specimens examined: D. Sacc. Myc. Ital. *978*, on leaves of *Rhododendron hybridum* and *R. ponticum*, Italy, sub *P. versicolor* Speg. var. *rhododendri* D. Sacc.; on blighted leaves of *Rhododendron obtusum* var. *amoenum*. Jamaica, Long Island, N. Y., Sept. 1924, P. E. Case.

This species differs from *P. macrotricha* Klebahn by the broader and shorter spores, the shorter setae, and the strongly contrasted color of the median cells.

16. PESTALOTIA GIBBOSA Harkness, Bul. Calif. Acad. Sci. **2**: 439–440. 1887.

Acervuli epiphyllous, sparse, rarely confluent, punctiform, erumpent, black, sub-globose, 70–140 μ wide, seated on pale, dead spots or dead areas with definite borders.

Conidia 5-celled, unequilateral, constricted at septum dividing the 2 lower colored cells, 25–30 μ; median colored cells 16–19 × 9–10.5 μ, the upper 2 fuliginous and subglobose, the lower olivaceous, sub-cylindric; end cells hyaline, the basal cell long conic, or somewhat tapering into a short, filiform pedicel up to 6.4 μ long, sometimes knobbed at the base; apical cell, long cylindric, bearing 2–4, usually 3, long, curved setae, 30–60 μ, coarse and widely divergent, often with capitate or knobbed extremities (Fig. 5, *16*).

On pale, dead spots and areas of living leaves of *Gaultheria shallon*, Seattle, Washington, Dec. 1893, C. V. Piper. The type of this species has not been available, but the original description of *P. gibbosa* agrees with the fungus studied. According to Harkness, the type collected at Point Reyes, California, June, 1886, and distributed in Pacific Coast F. *4130* frequently covers nearly the entire leaf, which is blackened with spores.

17. PESTALOTIA LEPROGENA Speg. Anal. Mus. Nac. Buenos Aires **23**: 119. 1912.

Spots repand-orbicular, somewhat large, separate, at length confluent, broadened irregularly, dark, leathery.

Acervuli loosely gregarious or scattered, black, conoid, erumpent, circinate, often confluent, sooty on maturity, 72–145 μ wide, blackening the surface in places.

Spores 5-celled, clavate, tapering to the base, curved or erect, 18.8–22.2 μ; median cells guttulate, colored, the 2 upper fuliginous, opaque, swollen, the lower olivaceous, 13–15.5 × 7.7–8.8 μ; end cells hyaline, large, broad conic, setae 3, slender, spreading, 15–26.6 μ, widely divergent; pedicel fragile, 4.5–7.7 μ (Fig. 6, *21*).

On mature fruits of *Musa sapientum* and *M. pedicellata* ex Brasilia, Buenos Aires and La Plata, Nov. 1911, Spegazzini.

Pustules are seated on spots which at first are 2–5 mm. in diameter, later larger, dirty in color and leathery, often covering the entire fruit, deforming and discoloring it. Stevens, Fungi of Porto Rico *122* sub *P. funerea* on leaves of *Musa paradisiaca*, differs from the type only in the length of the setae which measure 10–15.5 μ and in the lighter color of the upper two of the three median cells. The acervuli are largely epiphyllous, circinate, sooty at maturity, effuse, seated on large white spots or areas with dark brown or purple borders.

18. PESTALOTIA PALLIDICOLOR Speg. Ann. Mus. Nac. Buenos Aires III. **13**: 412. 1911; Sacc. Syll. Fung. **22**: 1227. 1913.

Acervuli epiphyllous, small, sparse, scattered, at first covered then erumpent, circinate, black, 72–125 μ wide, distinct.

Conidia erect, elliptic-fusoid, 16.5–20 μ, not, or only slightly, constricted at septa; median cells guttulate, pale olivaceous, equally colored, 12.2–13.3 × 5.5–6.6 μ; exterior hyaline cells short conic, basal obconic, apical cell mucroniform; setae 3, short, 8.8–15 μ; pedicel 2.2–6.6 μ, abruptly expanding into the basal cell (Fig. 5, *15*).

On dry or decaying scapes of *Synandrospadix vermitoxica*, Botanic Gardens, La Plata, Argentina, Oct. 1910, Spegazzini.

19. PESTALOTIA GRACILIS, Klebahn, Myc. Centralbl. **4**: 10–11. 1914. *P. sassafras* E. & E. nomen nudum, Ellis No. Amer. Fung. *1376*. 1885.

Fruiting bodies amphigenous, mostly hypophyllous, lenticular, subepidermal then erumpent, surrounded by the torn epidermis, 60–200 μ in diameter, densely gregarious and confluent, blackening the matrix, spores issuing in black cirrhi.

Spores 5-celled, erect, fusoid, sometimes slightly curved, slightly constricted at septa, 19.5–23 μ; lower median cell olivaceous, the upper 2 umber, 12–15 × 6–7 μ; basal hyaline cell long conic, tapering, apical cell large conic or cylindric bearing a crest of 2–4, usually 3, widely divergent setae, 13–25 μ; pedicel erect, 4–7 μ (Fig. 5, *14*).

On dead dried leaves of *Sassafras variifolia*. Sooty mats of spores cover both surfaces of the brown leaves. Study is based on type specimens in Ellis No. Amer. Fung. *1376*, on dead leaves of *Sassafras*, Newfield, N. J., Oct. 1884. Fungus is also distributed in E. & E., Fung. Col. *370b* on leaves of *Laurus sassafras* [= *Sassafras variifolium*], Newfield, N. J., Oct. 1883, sub *P. guepini* Desm.

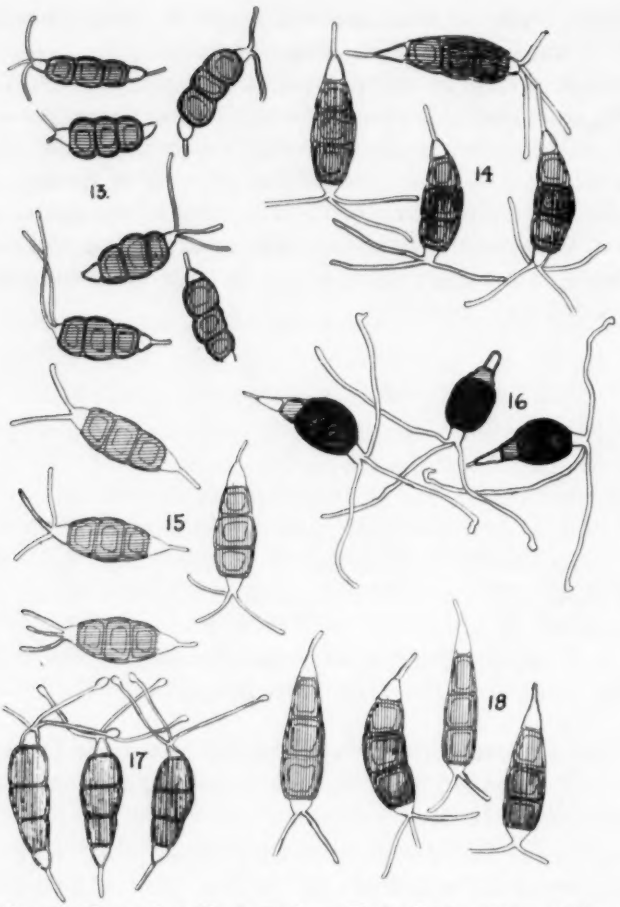

FIG. 5. Conidia: *13, P. torulosa* B. & C. from Herb. Berk. *5055*, at Kew, on seeds of *Citrullus vulgaris* So. Carolina; *14, P. gracilis* Klebahn from Ellis, No. Amer. Fung. *1376*, on leaves of *Sassafras*, sub *P. sassafras* E. & E.; *15, P. pallidicolor* Speg., on *Synandrospadix vermitoxica*, La Plata, Argentina, 1910, Spegazzini; *16, P. gibbosa* Harkness on *Gaultheria shallon*, Seattle, Washington, Dec., 1893, C. V. Piper; *17, P. clavata* Cke. & Ellis, No. Amer. Fung. *350*, on living leaves of *Smilax*, Sept., 1879; *18, P. copernica* Speg., on decaying fruits of *Copernica cerifera*, Puerto Leon, Argentina, 1909, Spegazzini. ×750.

Klebahn described *P. gracilis* on the basis of several different forms from unrelated hosts of which E. & E., Fung. Col. *370b* is the first listed. This specimen and the type of *P. sassafras* E. & E. are identical. A description of *P. sassafras* was never published; consequently the name is reduced to synonymy.

20. PESTALOTIA MENEZESIANA Bres. and Torr. Broteria (Bot.) **8**: 142. 1909; Sacc. Syll Fung. **22**: 1222. 1913.

Acervuli amphigenous, black, numerous or densely gregarious and coalescing freely, effuse and spreading over the surface, at first subepidermal, then erumpent, surrounded by the torn epidermis, 80–160 μ in diameter.

Conidia 5-celled, curved, broad at the top, tapering into a rather long conical basal cell, 16.8–21.6 μ; median cells differently colored, the 2 uppermost fuliginous, somewhat cask-shaped, the lower olivaceous, slightly constricted at the septa, 12–14.4 × 6.4–7.2 μ; basal cell long conic, apical cell narrow cylindric, often long; crest of 3 setae, 16–30 μ, divergent; pedicel short, erect, up to 5 μ (Fig. 3, *19*).

Occurring as a saprophyte on dried leaves of *Vitis vinifera*. Torrend, Fung. Sel. Ex. *185*, on leaves of *Vitis vinifera*, Porto Santo, Madeira Islands, A. de Noronha.

21. PESTALOTIA COPERNICA Speg. Anal. Mus. Nac. Buenos Aires, III. **13**: 411. 1911; Sacc. Syll. Fung. **22**: 1227. 1913.

Acervuli lenticular, conoid, 75–175 μ in diameter, circular, scattered or gregarious, freely confluent, forming black incrustations, subepidermal at first, then erumpent, generally distributed and darkening the matrix.

Conidia clavate, 5-celled, erect or sometimes slightly curved, tapering to the base, not, or hardly, constricted at the septa, 20–24.5 μ; median cells guttulate, olivaceous, the upper 2 sometimes slightly darker than the lower, 15.5–17.7 × 5.5–6.6 μ; end cells hyaline, the apical cell usually short conic bearing 2 or rarely 3 short, straight setae, 6–12 μ, divergent, basal cell hyaline, long, tapering into a short pedicel, 2.5–5 μ (Fig. 5, *18*).

On somewhat decayed, immature fruits of *Copernica cerifera*, existing as a saprophyte, Puerto Leon, Misiones, Argentina, June, 1909, Spegazzini.

22. PESTALOTIA CLAVATA Cke. & Ellis, Grevillea **6**: 85, pl. 99, fig. 7, 1878; Sacc. Syll. Fung. **3**: 796. 1884; Voglino, Sul. Gen. Pest. pl. 9, fig. 17. 1886.

P. grandis B. & C. in Crié, Ann. Sci. Nat. Bot. VI, **7**: 59, pl. 8, fig. 21, 1878, 1879. Voglino, Sul. Gen. Pest. p. 32; Sacc. Syll. Fung. **10**: 491. 1892. Curtis Geol. and Nat. Hist. Survey No. Car. Bot. III, 118. 1867. nomen nudum.

Acervuli numerous, crowded, 100–175 μ in diameter, amphigenous, sometimes entirely epiphyllous, lenticular, sub-epidermal then erumpent, seated on brown concentrically zoned spots with purple margins.

Spores fusiform, usually slightly curved, 5-celled, 25–31 μ, slightly constricted at septa; colored cells umber, sometimes olivaceous, equally colored,

16–21 × 7–8.5 μ; end cells hyaline, prominent, apical cell long cylindric, bearing 2–3 (usually 3) curved divergent setae; basal cell broad conic with a straight pedicel, 2.2–7.5 μ; setae 9–19 μ with a conspicuous knob at the extremities (Fig. 5, *17*).

On living leaves of *Smilax spp.* causing definite leaf spots. Specimens examined: Curtis Herb. *4046*, Alabama, from Peters *437*, sub *P. grandis* B. & C. on living leaves of *Smilax;* Ellis No. Amer. Fung. *350*, on living leaves of *Smilax*. Sept. 1879; Barth. Fungi Col. *4247*, on leaves of *Smilax rotundifolia*, Shreveport, La., Oct. 16, 1913, E. Bartholomew, associated with *Phyllosticta smilacis* E. & M.; Ellis, Fungi of New Jersey *2740*, on leaves of *Smilax*, Newfield, N. J., J. B. Ellis; Herb. S. M. Tracy *5242*, on leaves of *Smilax laurifolia*, Ocean Springs, Miss., S. M. Tracy, 5–2–1898, sub *P. funerea* Desm.; Herb. N. Y. State Museum, on living leaves of *Smilax glauca*, Babylon, Long Island, N. Y., Sept. 19, 1915, sub *P. guepini* Desm.

The original description of this fungus by Cooke and Ellis mentions 3-celled spores although the accompanying illustration shows 5 cells and 3 setae. Voglino (87) found 4-septate spores and setae with knobbed extremities in the type specimen.

This species was originally named *P. grandis* B. & C. by Curtis, but Berkeley, who described many of Curtis' specimens, failed to publish a description for it. Crié (20) cited this species and published an illustration of its spore which, however, offers an incorrect interpretation of *P. grandis*. Voglino listed *P. grandis* without its host or the source of the specimens. He furnished a brief description based on Crié's figure of the spore which, however, is untenable. Crié undoubtedly obtained type specimens from Berkeley, but since the description of *P. clavata* Cke. & Ellis antedates Crié's publication, *P. grandis* becomes a synonym.

23. PESTALOTIA STELLATA, B. & C., Grevillea **2**: 155. 1874. Sacc. Syll. Fung. **3**: 787. 1884.

Acervuli stellate, black, punctiform, sparse, amphigenous, flat, 140–280 μ in diameter, seated on definite pale, circular spots or irregularly shaped dead areas with black borders.

Spores 5-celled, erect or sometimes slightly curved, broad fusiform or broad elliptic, only slightly constricted at septa, 17.7–20 μ; 3 median cells cask-shaped, guttulate, 12–15.5 × 6.6–8.8 μ, the 2 upper colored umber, the lower olivaceous; end cells hyaline, small, short conic, the apical one bearing a crest of 3 broadly divergent setae, 4.4–11 μ; pedicel attenuated downwards (Fig. 6, *20*).

Pale definite spots on living leaves of *Ilex opaca*, usually secondary and associated with other fungi. Specimens examined: Herb. Curtis *4921* at Harvard, Society Hill, So. Carolina, Apr. 1855, and similar specimens in

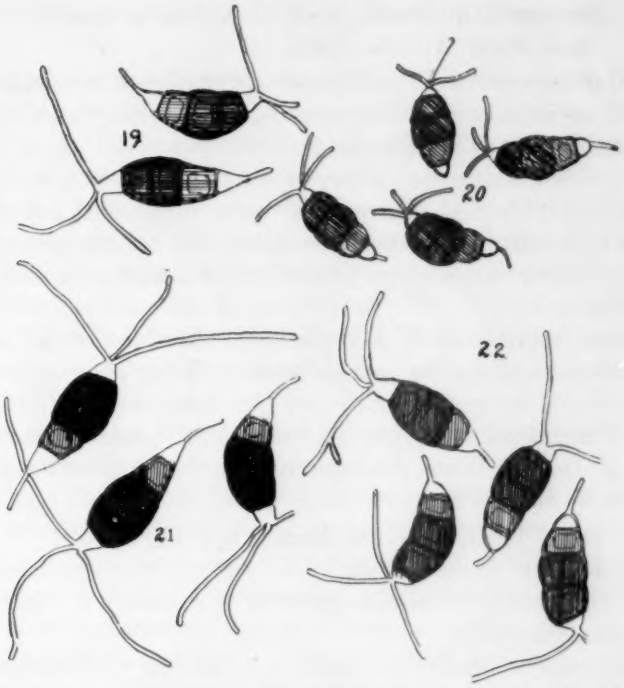

FIG. 6. Conidia: *19, P. menezesiana* Bres. & Torr., on *Vitis vinifera*, Madeira Islands; *20, P. stellata* B. & C. from Herb. Berk. *4921*, at Kew, on *Ilex opaca*, So. Carolina; *21, P. leprogena* Speg. on *Musa sp.*, La Plata, Argentina, Spegazzini; *22, P. rhododendri* (Sacc.) Guba from D. Sacc. Myc. Ital. *978*, on leaves of *Rhododendron hybridum* and *R. ponticum*, sub *P. versicolor* Speg. var. *rhododendri* D. Sacc. ×750.

Berkeley's herbarium at Kew; Myc. Coll. U. S. D. A., Liberty, Texas, Nov. 6, 1914, H. Jolivette sub *P. funerea.*

Cooke and Ellis (Grevillea **6**: 85. 1878) reported this species on *Quercus tinctoria* in Ellis, Fungi of New Jersey *2640*, and Saccardo (Syll. Fung. **3**: 787. 1884) subsequently cited both hosts. The form on *Quercus* lacks the pale white spots and contrary to the opinion of Cooke and Ellis is distinct from *P. stellata* B. & C. The fungus in Ellis No. Amer. Fung. *1352* labelled *P. stellata* on leaves of *Quercus tinctoria* and *Vaccinium arboreum* is not this species. According to Voglino (87), specimens of *P. stellata* in Ravenel, Fung. Amer. Ex. *254* on leaves of *Nerium* and in E. & E., No. Amer. Fung. *34* on leaves of *Ilex opaca* belong to this species. Both are different species, the latter is *P. annulata* B. & C. and the former a new species.

24. PESTALOTIA TORULOSA, B. & C. Grevillea **2**: 155. 1874; Sacc. Syll. Fung. **3**: 795. 1884.

Acervuli minute, scattered and frequently confluent, sub-epidermal, then exposed, flat or usually round, numerous, black, punctiform, 72–190 μ in diameter, usually seated on black spots.

Spores 5-celled, clavate, torulose, or strongly constricted at the septa, usually curved, 17.5–20 μ; the colored cells olivaceous, guttulate, 13.3–15.5 × 5.5–6.6 μ; end cells hyaline, the apical one cylindric, the basal cell long conic tapering into a short pedicel about 2.2 μ; setae 2–3 divergent 6.6–11 μ (Fig. 5, *13*).

On seeds of watermelon, *Citrullus vulgaris*, So. Carolina in Curtis Herb. *5035* at Harvard and similar specimens in Berkeley's herbarium at Kew.

25. PESTALOTIA VIRGATULA Klebahn, Myc. Centralbl. **4**: 13. 1914. *P. funerea* Desm. *f mangiferae* Sacc. Atti Istit. Veneto Sci. VI, **2**: 461. 1884.

Pustules small, black, scattered, largely hypophyllous.

Conidia 5-celled, clavate, 18–22 × 6.5–7.8 μ, the two upper colored cells umber or fuliginous, lower olivaceous; setae 3 or rarely 2, relatively stout, 18–21 μ, widely divergent.

On dead leaves of *Mangifera indica* in herbarium of A. Brunaud, specimen of which carries the name "*Sphaerella tahitensis* in association with *P. funerea*. On leaves of Manguier, Tahiti, A. Brunaud."

The fungus shows no relation to *P. funerea* Desm. Klebahn evidently was unaware of a published description of this fungus. His erection of the new name and the segregation of the fungus from *P. funerea* Desm. was correct.

26. PESTALOTIA VERSICOLOR Speg., Michelia **1**: 479. 1879; Sacc. Syll. Fung. **3**: 790. 1884.

Pustules sparse, globose-lenticular, at first covered then erumpent, raised, surrounded by shreds of the torn epidermis and cuticle, 80–215 μ wide, on maturity discoloring the surrounding matrix.

Conidia 5-celled, clavate, erect or somewhat curved, only slightly constricted, 22–26.5 μ; median cells colored, guttulate, the two uppermost cask-shaped, fuliginous, the lower olivaceous, 15–17.7 × 7.8–8.8 μ; end cells hyaline, prominent, the apical one usually short cylindric and bearing 3, rarely 4, coarse widely divergent setae, 17.7–26.5 μ; basal cell long and broad conic often inflated, descending into a short straight pedicel, 4–7 μ (Fig. 7, *24*).

On fallen decaying leaves of *Nerium oleander*, Conegliano, Italy, 1876, Spegazzini.

The entire matrix is colored pale gray and the black pustules are distributed without order. Other fungi are present. Klebahn (43) included all 5-celled spore forms with median cells of contrasted color in *P. versicolor*. In these forms considered, the upper two median cells are dark in contrast to the lower olivaceous colored cell. The opaque cells are cask-shaped or sub-globose. Within the species *P. versicolor*, Klebahn has included many forms from different hosts, based upon Saccardo's compilation of this species and his study of certain exsiccati. Type specimens were not considered. Specimens in D. Sacc. Myc. Ital. *978* named *P. versicolor* var. *rhododendri* on leaves of *Rhododendron hybridum* and *R. ponticum* and in Barth. Fung. Col. *2441* named *P. guepini* on *Ribes rubrum* are considered by Klebahn to belong to *P. versicolor*. These forms according to my studies of the types do not belong to this species.

Klebahn (42, 43, 14–16) successfully infected a wide range of plants with a form from *Darlingtonia* which he determined to be *P. versicolor*. From this fact he assumed that the fungus was of world-wide distribution. His knowledge of the fungus was based on the original description which lacks many details necessary to a good understanding of the species. His form on *Darlingtonia* has spores measuring 26–31 μ while those of the type measure 22–26.5 μ. Klebahn's conception of the type is incorrect and his form on *Darlingtonia* belongs elsewhere.

Klebahn was able to infect *Darlingtonia* under bell-jars but not in open greenhouses. He asserted that this form was essentially a saprophyte capable of existing under moist conditions on different substrata but not causing infection of living or freshly wounded tissue.

27. PESTALOTIA MOLLERIANA Thümen, Myc. Univ. *1988*, 1881.

Pustules amphigenous elevated, usually scattered and solitary, circular, densely gregarious and confluent, at first subepidermal later erumpent, freed, blackening the matrix, tearing the epidermis irregularly, 140–280 μ wide, without spots.

Conidia fusiform, narrowed at the ends, usually erect, equilateral, 5-celled, only slightly constricted at septa, 19–22 × 6.3 μ; median cells guttulate, olivaceous, equally colored, 12.7–14.9 μ; end cells hyaline, basal one broad conic, abruptly contracted into a short stalk, 4–7 μ, apical cell conic or cylindric with a crest of 2–3 setae, 10–16 μ, widely divergent, curved (Fig. 7, *23*).

On fallen dried leaves of *Eucalyptus sp.* Oct. 1879, Choupal, Portugal, Möller in Thümen, Myc. Univ. *1988* and Roumeguère Fung. Sel. Gall. Ex. *5167*. Thümen stated that the spores are 5-septate. My study shows that they have 5 cells.

28. PESTALOTIA AQUATICA E. & E., Jour. Myc. **5**: 157. 1889; Sacc. Syll. Fung. **10**: 492. 1892.

Spots amphigenous, chestnut brown, definite, irregular in shape and size, as much as 3 cm. long and 2 cm. wide, concentrically wrinkled, surrounded by a purplish or dark border. Acervuli epiphyllous, sparse, scattered, erumpent, circinate, black, 80–130 μ in diameter, raised, convex then concave.

Conidia 5-celled, obovate, erect or slightly curved, not constricted at the septa, 20–24 μ; median cells guttulate, olivaceous, the upper 2 somewhat darker, the lower lighter in contrast, 13–15.6 × 7.2–8.4 μ; end cells hyaline, short conic, the apical one with a crest of 3 stout setae 10–20 μ, unequal in length and widely divergent; pedicel erect, 2.4–4 μ (Fig. 7, *26*).

Occurring as a saprophyte on living leaves of *Peltandra virginica*, Newfield, N. J., Aug. 27, 1889, Myc. Coll., U. S. D. A.

29. PESTALOTIA VERMIFORMIS Massee, Kew Bul. Misc. Inform. **1901**: 156. 1901; Sacc. Syll. Fung. **18**: 479. 1906.

Acervuli amphigenous, largely hypophyllous, solitary and scattered or crowded and confluent, subepidermal then erumpent, black contents issuing in cirrhi, present over entire leaf surface, 60–210 μ in diameter.

Spores 5-celled, erect often slightly curved, hardly constricted, clavate, tapering toward the base, 17.5–20 × 5.5–6.2 μ; 3 median cells olivaceous, equally colored, guttulate, 11–13.7 μ; end cells hyaline, the apical one cylindric or conic with 2–3, usually 3, simple setae, broadly divergent, 11–16 μ, basal cell long conic, tapering, pedicel erect, 2.2–5 μ (Fig. 7, *25*).

On living leaves of *Eugenia sp.*, Mt. Cook, Endeavour River, Queensland, Bailey, in Kew herbarium bearing the number *1132*. The pustules are present generally over the leaf surface. Massee stated that the pustules are epiphyllous and that the spores are 4–5 septate. Examination of type material shows that they are largely hypophyllous and that the pustules are not confined to pallid spots. The spores are not 25–32 μ long as the type description indicates and the septa are uniformly four. Spores with four median colored cells were not observed.

30. PESTALOTIA INQUINANS Cke. & Harkness, Grevillea **12**: 94. 1884; Sacc. Syll. Fung. **3**: 784. 1884.

Acervuli amphigenous, largely epiphyllous, numerous, 140–280 μ in diameter, subepidermal then erumpent, surrounded by the torn epidermis, coalescing freely and forming large spore cushions, the contents on maturity spreading over the matrix, leaving sooty deposits.

Spores 5-celled, erect, clavate-fusoid, slightly constricted at the septa, 22.2–26.6 μ; median cells guttulate, dark olivaceous, equally colored, 15.5–

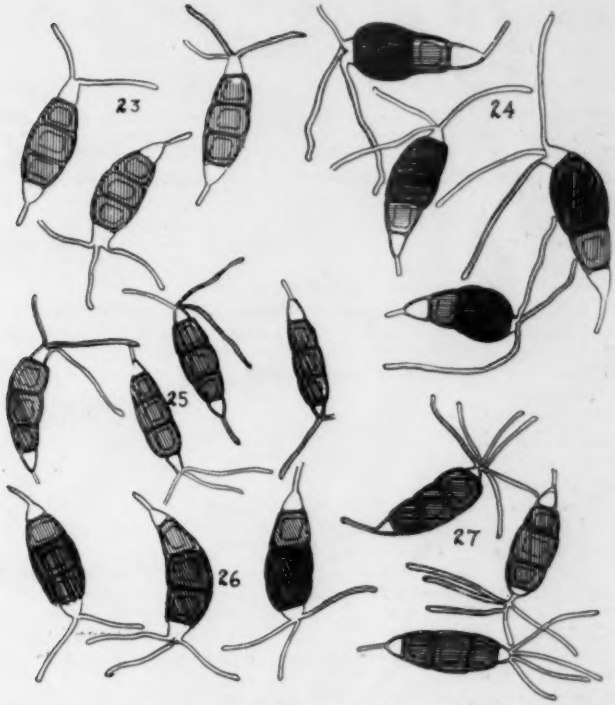

FIG. 7. Conidia: *23, P. molleriana* Thüm., from Thüm. Myc. Univ. *1988*, on leaves of *Eucalyptus sp.; 24, P. versicolor* Speg. on *Nerium oleander*, Conegliano, Italy, 1876, Spegazzini; *25, P. vermiformis* Massee, from Kew *1132*, on *Eugenia sp.*, Mt. Cook, Endeavour River, Queensland, Bailey; *26, P. aquatica* E. & E., from Myc. Coll., U. S. D. A., on *Peltandra virginica*, Newfield, N. J., 1889; *27, P. inquinans* Cke. & Hark. from Kew *2094*, on *Eucalyptus*, California. ×750.

17 × 7.5–8.8 μ; end cells hyaline, the apical one conic or short cylindric bearing a crest of 4–6, usually 4 or 5, curved setae, 11–20 μ, extending forward or sidewards; basal cell large conic with a short pedicel, 4.4–8.8 μ (Fig. 7, *27*).

On fallen dead leaves of *Eucalyptus sp.*, California, in Kew herbarium bearing the number *2094*.

The pustules are present on both leaf surfaces, densely gregarious in places, forming sooty deposits. The presence of numerous setae are characteristic of the spores of this species. The fungus exists in association with other fungi.

KEY TO SPECIES INCLUDED IN PART I

The usefulness of this key is limited to those species of *Pestalotia* listed in the species index. In using this key, mean dimensions and typical characters should be emphasized. Aberrances should not be considered.

Conidia 5-septate; fruiting body an acervulus seated on a stroma, usually pezizoid	*P. pezizoides* de Notaris
Conidia 4-septate; fruiting bodies either acervuli or pycnidia, stroma lacking	
Colored cells 10–16 μ long	
Conidia 4.8–6.6 μ wide	
Setae 1–4, frequently branched	*P. guepini* Desm.
Setae 3, sometimes 2, rarely branched	
Setae 6–11 μ long, conidia torulose	*P. torulosa* B. & C.
Setae 9–20 μ long, conidia not torulose	
Setae knobbed at extremities	*P. palmarum* Cke.
Setae not knobbed	
Colored cells very pale	*P. pallidicolor* Speg.
Colored cells olivaceous; pustules circular	
Conidia elliptic-fusoid, about 6.5 μ wide	*P. molleriana* Thüm.
Conidia narrow-fusoid, 5.5–6.2 μ wide	*P. vermiformis* Massee
Colored cells umber, pustules elongate	*P. foedans* Sacc. & Ellis
Upper two colored cells umber, pustules circular	*P. gracilis* Klebahn
Setae 20–30 μ long	*P. vaccinii* (Shear) Guba
Conidia, 6.4–10.5 μ wide	
Setae 4–11 μ long	*P. stellata* B. & C.
Setae 10–30 μ long	
Apical cell long, cylindrical or conic; basal cell long, conic or tapering	
Upper two colored cells umber, lower olivaceous	*P. gracilis* Klebahn
Upper two colored cells fuliginous	
Conidia 6.4–7.2 μ wide	*P. menezesiana* Bres. & Torr.
Conidia 7.7–8.8 μ wide	*P. leprogena* Speg.
Apical cell short, cylindric or conic; basal short, obtuse	
Colored cells olivaceous or upper two slightly darker than lower	*P. aquatica* E. & E.
Upper two colored cells fuliginous	
Pustules elongate, concentrically arranged	*P. curta* Sacc.
Pustules circular, punctiform	
Conidia obovate	*P. cliftoniae* Tracy & Earle
Conidia clavate	*P. virgatula* Klebahn
Colored cells 15–18 μ long	
Conidia 5.5–6.6 μ wide	*P. copernica* Speg.
Conidia 6.5–9.5 μ wide	
Setae 5–20 μ long	
Colored cells olivaceous, conidia elliptic-fusoid	*P. inquinans* Cke. & Hark.
Colored cells umber, conidia clavate	*P. funerea* Desm.
Upper two colored cells fuliginous, conidia obclavate	*P. glandicola* (Cast.) Guba
Setae 18–27 μ long, not knobbed; colored cells fuliginous	
Pustules hysteriform	*P. scirpina* Ellis & Martin
Pustules circular	
Conidia 7.8–8.8 μ wide	*P. versicolor* Speg.
Conidia 8.5–9.5 μ wide	*P. sphaerelloides* Ellis & Langl.
Setae 25–48 μ long, knobbed; colored cells olivaceous	*P. theae* Sawada
Colored cells 16–23 μ long	
Conidia 7–10.5 μ wide	
Setae 7–19 μ long	
Setae usually 3, knobbed	*P. clavata* Cke. & Ellis
Setae usually 5, frequently knobbed	*P. cesatii* Rabenhorst

Setae 17–60 μ long	
Colored cells olivaceous, equally colored or upper two slightly darker	
Setae knobbed	*P. theae* Sawada
Setae not knobbed	*P. macrotricha* Klebahn
Upper two colored cells fuliginous	
Conidia elliptic-fusoid	*P. rhododendri* (Sacc.) Guba
Conidia obclavate	*P. gibbosa* Hark.

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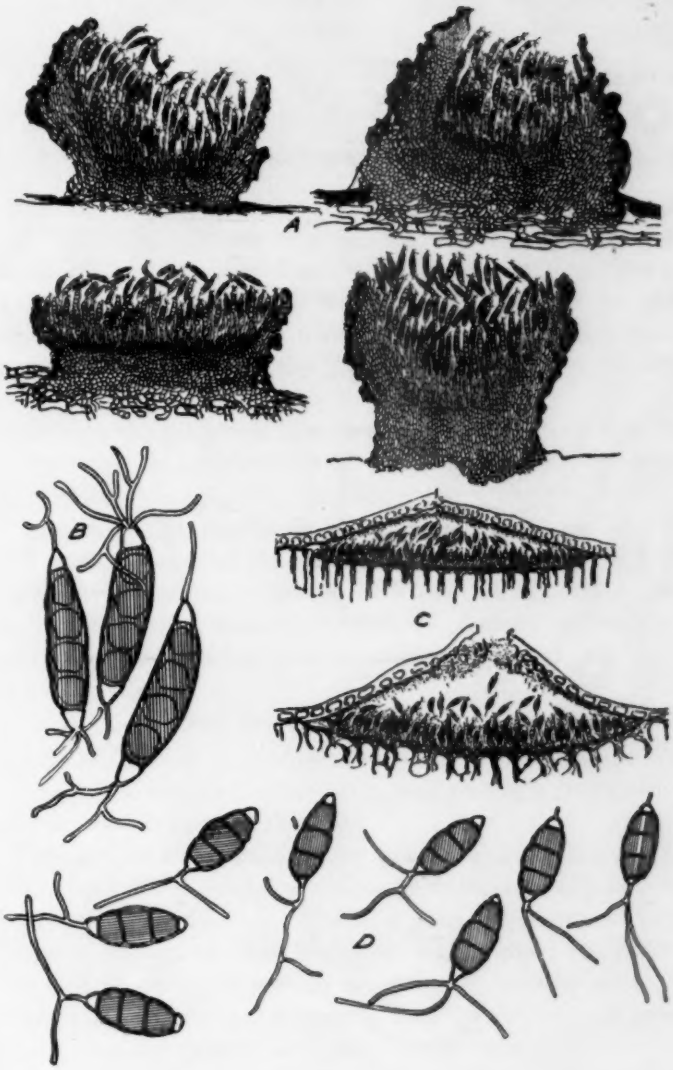
EXPLANATION OF PLATE I

A. Section through fruiting pustule of *P. pezizoides* de Notaris from Ellis, No. Amer. Fung. *35*, on canes of *Vitis vinifera*.

B. Conidia of *P. pezizoides* de Notaris from Michener Fungi **19**: 82, *2790*. Myc. Coll., U. S. D. A., on canes of *Vitis sp.* not *Humulus lupulus*, sub *P. compacta* B. & C. ×750.

C. Sections through fruiting pustules of *P. guepini* Desm. from Myc. Coll., U. S. D. A., on leaf of *Camellia japonica* in greenhouse, Washington, D. C., May, 1907, W. A. Orton, sub *P. camelliae* Pass.

D. Conidia of *P. guepini* Desm. from Desm. Pl. Crypt. Fr. Fasc. 22, *1084*, on leaves of *Camellia japonica*. ×750.


A
B
C
D

THE EFFECT OF THE H-ION CONCENTRATION OF THE CULTURE SOLUTION ON THE BEHAVIOR OF FUSARIUM CROMYOPHTHORON AND ALLIUM CEPA AND THE DEVELOPMENT OF PINK-ROOT DISEASE SYMPTOMS[1]

C. P. Sideris

INTRODUCTION

The pink-root disease of onions is of great importance to the onion growers of California. Official reports (40) estimate field losses at 14 per cent without taking into consideration, at the same time, storage losses which have resulted from the same trouble. The disease is not confined to California, but has a very wide distribution, having been reported from every onion-growing section in the United States, Canada, Australia, and the Bermuda Islands.

All reports on the pink-root disease point out definitely that the causal parasite is an organism belonging to the genus *Fusarium*. Taubenhaus and Mally (37), of the Texas Agricultural Experiment Station, who have made extensive studies on the occurrence of the pink-root disease in Texas, have determined and also named the pathogenic organism as *Fusarium malli* Taub. This contribution as a whole is a compilation of careful observations and experimental results concerned with the factors influencing the development of this disease under field conditions.

THE DISEASE IN CALIFORNIA

The pink-root disease occurs in more than one locality in California. The writer has often received onions with pink-root symptoms sent by farm advisors from various parts of the state.

One of the largest onion-growing sections of California is the Delta district of Stockton. This district is composed mainly of a number of small islands in the Sacramento river. The soil consists almost entirely of peat derived from a vegetation that flourished in the past, representatives of which may still be found in certain uncultivated marshy areas. The underground system of irrigation is practiced by the majority of growers, this proving to be very inexpensive and extremely satisfactory.

In spite of the uniform conditions that one finds in fields of this type, with respect to soil texture, organic matter, and distribution of water there are areas, in any given field, in which the chemical properties differ greatly,

[1] Thesis submitted to the Graduate Division of the University of California in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

although the physical properties of the soil may be relatively uniform. Such differences may be found more often in the acidity and salt concentration of the aqueous extract of the soil. The differences in the acidity of different soil samples may run as high as 2.0 pH values. It was particularly noticeable in certain fields that had not been leveled perfectly that the pH value of the aqueous extracts of the soil taken from the higher spot was greater than that taken from the lower spot. Such differences in pH value may be attributed to differences in the concentration of salts resulting from differences in the rate of evaporation of water.

The physicochemical differences of the aforesaid localized field areas were reflected in the growth and condition of the plants grown thereon. The plants on the high-leveled areas were slow growing and unhealthy, whereas those on the low-leveled areas were very luxuriant and healthy. This striking coincidence of soil conditions with poor plant development led the writer to suspect that soil conditions might be partly, if not wholly, responsible for the weakened and unhealthy condition of the plants. The factors that may be responsible for the development of this condition are: (a) soil acidity, (b) relative salt concentration, and (c) rapid changes in the moisture content of the two different field areas. Other causes believed to operate concurrently with the above are parasitic or facultative microorganisms. Examinations of the roots of many such plants proved that the most frequently occurring microorganisms were species of the genus *Fusarium*.

PATHOLOGICAL AND CULTURAL TECHNIQUE

Isolation of Microorganisms from Root Tissues

The technique employed for the isolation of the various microorganisms associated with tissues of diseased roots was as follows. The root or roots were first thoroughly washed with ordinary tap water, then transferred to sterilized tap water, with which they were washed as much as possible until all soil particles had been removed, and then placed between sheets of sterilized filter paper for dehydration. At the end of this operation the root, or roots, selected for examination were put in a sterile petri dish where they were sectioned by sterile instruments into very small pieces. Each piece was transferred into a petri dish containing nutrient agar. The nutrient agar used for this purpose was prepared in three distinct lots, each one with a different degree of acidity. Lot A had a reaction of pH 4.0; B, 6.8; and C, 7.5. The main reason for adopting such a procedure was to encourage or discourage the growth of various micoorganisms. This method of isolation yielded many microorganisms, the majority of which were ordinary saprophytes of the soil. Beside these, some species of

Fusarium, a *Rhizoctonia*, a *Phoma*, a *Cephalosporium* and a number of species of *Penicillium* and *Aspergillus* were obtained. Fusarium organisms were, however, invariably associated with diseased tissues; for which reason their possible relationship to pink-root disease was studied. A certain species of *Fusarium*, occurring quite often in diseased tissues in between 30 and 40 per cent of the cases, was selected for these studies.

REVIEW OF PREVIOUS WORK

The pink-root disease of onions is a comparatively new disease, and the literature associated with it is relatively meager. The first report of its occurrence in the United States was made in 1913, but no studies were undertaken until 1917. The first real contribution is that of Taubenhaus and Mally (37).

A number of species of *Fusarium* have been studied in the past by various investigators in connection with many root-rot and wilt diseases on a variety of plants. Nearly all these studies are concerned more with moisture and temperature, as factors contributing to the development of root-rot or wilt diseases, than with the relative acidity of the soil solution. There are a number of excellent researches on the physiological behavior of Fusarium spp. in a number of culture media at different H-ion concentrations. Although some of these researches give a very good idea of the behavior of Fusarium spp. when in contact with a number of organic substances, most of them have produced no information on the parasitic potentiality of the organisms when in contact with living tissues of different hosts.

The studies of Young and Bennett (46) on *Fusarium oxysporum* Scht. in culture media at different H-ion concentrations give the behavior of this fungus as follows: ". . . the reaction of the culture solution in which *F. oxysporum* was grown continued to become acid until an H-ion concentration of pH 3.6 was reached, then turned toward alkalinity, and growth continued until all the organic compounds were broken up and an H-ion concentration of pH 8.4 was reached." MacInnes (24), in a similar study on a wheat scab *Fusarium*, found that ". . . as growth proceeds, the H-ion concentration of the solutions is changed by the organism, those on the acid side tending to become more alkaline and those on the alkaline side more acid." Johnson (16), in studying seven soil molds, including *Fusarium bullatum* and *oxysporum*, found that ". . . the optimum reaction for the molds lies more to the acid side of the neutral point than toward the alkaline, practically all of them making nearly a maximum growth as low as pH 2.0, while they are cut off quite sharply at pH 8.7. . . . The reaction of all the solutions was changed toward a more alkaline value except the

neutral solution with *F. bullatum*. . . . In no case did the molds reach the pH values which were found to inhibit their growth in the studies on the effect of hydroxyl ions." Webb (42), in studying the germination of spores of a species of *Fusarium*, at different H-ion concentrations, found that ". . . the range of the spore germination with respect to the reaction of the medium is between pH 2.0 and pH 10.0. Maxima generally appear between pH 3.0 to pH 4.0 and near pH 7.0." Karrer (19), in determining the action of amylase secreted by a species of *Fusarium* at different H-ion concentrations, found that ". . . the maximum total accumulation was produced in the solution having an initial pH of 4.5 and a final pH of 7.8." Hopkins (15), in his studies on *Gibberella saubinetii* Sacc. in its relation to the wheat-scab disease, found that ". . . the fungus has two maxima, one on the acid and the other on the alkaline side. The maximum rate of infection coincides with the maximum growth of the organism." Johnson (17) reports as follows on the effect of acidity on the Fusarium wilt of tobacco: "In one experiment all plants died at the three highest soil acidities, one died in each of the next three lower reactions and none died in the three jars at the alkaline end, although finally they all became infected. In another experiment all plants died in the first five grades of reaction from the acid." He furthermore states that . . . "the evidence seems fairly conclusive that an acid soil favors the wilt disease, although it may occur in neutral or alkaline soils." Rast (27) observed in fertilizer experiments that ". . . cotton wilt may be controlled with the use of fertilizers containing potash." Taubenhaus and Mally (37) state that *F. malli* may produce pink root disease in soils of both acid and alkaline reaction.

The behavior of Fusarium spp. in causing rot or wilt on a variety of plants at different temperatures has been studied by many investigators with excellent results. Link (20) found that the optimum temperature for the growth of *F. oxysporum* was 30° C., while that for *F. trichothecioides* Wr. was 12° C. Goss (7), using the same organism in studies on rot production, found that *F. oxysporum* grew more rapidly at 25° C. than *F. trichothecioides* and produced a more extensive rot on the potato tuber, the latter being able to produce a rot at much lower temperatures. Jones and Tisdale (18), in their studies on the influence of soil temperature on the development of flax wilt, found that "The minimum or critical soil temperature for the disease is about 14° C., with 38° C. as the maximum and the optimum for extreme disease development about 24° to 28° C." Tisdale (39), in similar studies, found that optimum temperature for growth and infective power of *F. lini* was 26°–28° C., with minimum 11° and maximum 34° C. Clayton (4), in his studies on the relation of soil temperature to the development of the Fusarium wilt of tomatoes, found that the

optimum temperature for the growth of the fungus was 28° C., and for the development of the disease 22°–32° C. Mann and Nagpurkar (21), in their studies on the Fusarium blights of potatoes in India, found that ". . . with an increase of temperature wilting of plants increases as well as rotting of tubers in storage." Dickson (5), in studying the influence of soil temperature and moisture on the development of the seedling blight of wheat and corn caused by *Gibberella saubinetii* found that ". . . the parasite functions normally over a comparatively wide range of temperatures, *viz.*, 3° to 32° C. The optimum temperature for spore germination, vegetative growth and sporulation is about 24° C. on unacidified and 28° C. on acidified media. The most favorable soil temperature for the blighting of wheat ranges from 12° to 28° C., while that for corn is from 8° to 20° C."

Moisture, as a factor influencing the production of rot in potatoes by species of *Fusarium*, has been studied by Goss (7), who found that ". . . the relative humidity plays a very important rôle in determining the progress of tuber rots, and has the same influence on all three species *viz.*, *F. oxysporum*, *F. radicicola*, and *F. trichothecioides*. With a high humidity at a given temperature the rotting was always greater than at a temperature 5° to 10° higher but with a low humidity." Wilcox, Link, and Pool (44) obtained a greater percentage of infection in cultures kept in saturated air than in others over Ca Cl_2.

The literature in connection with the physiological behavior of the onion plant is very meager. Rogers (29) states that onion plants require large amounts of moisture and moderate temperatures from the time of planting until they are mature.

In summarizing the results of the above workers one arrives at the following conclusions: (a) That species of *Fusarium* in culture media are able to increase or decrease the initial acidity or alkalinity of these media; (b) That the optimum temperature for the growth of these organisms and infection of their hosts lies at 20° to 30° C.; and (c) That species of *Fusarium* grow fairly well in moist media, and infection increases with increments of moisture plus temperature to a certain degree.

FUSARIUM SPECIES ASSOCIATED WITH ONION ROOTS

The number of Fusarium species that one may find associated with either healthy, dying, or dead roots is not definite. These organisms, being soil inhabitants, may be found on onions grown in acid as well as alkaline soil, in cold as well as in warm climate, and in moist as well as dry regions. The writer (32), in a survey of the Fusarium species associated with onion roots of plants grown in the Delta district, near Stockton, California, found that nearly 20 different species and varieties could be isolated from injured

or dead roots. Another *Fusarium* isolated from onion roots by Hanzawa (8) and Walker and Tims (41) is *F. cepae* Hanz. Hanzawa's organism, according to private information from Dr. Sherbakoff, is probably similar to that described by the writer (32) as *F. cromyophthoron* Sid. This organism, however, according to Hanzawa's description, either has not been investigated in connection with the development of pink-root symptoms or does not produce these symptoms, and, according to Walker's and Tim's statement, causes a dry to semi-watery decay of the scales with yellowing and premature dying of the tops. The prevailing opinion, therefore, as to whether *F. cepae* is identical with *F. cromyophthoron* is not definite.

The studies reported herein were confined to *F. cromyophthoron,* because of its constant association with onion roots showing pink root symptoms. A study of other species of *Fusarium* found on diseased tissues is being omitted at this time because of their less frequent association with the disease symptoms.

The name *F. cromyophthoron* is maintained throughout this presentation. This does not mean, however, that this organism is not closely related to, or identical with, *F. cepae.*

PHYSIOLOGICAL BEHAVIOR OF FUSARIUM CROMYOPHTHORON

Cultural Methods

Pure cultures of *F. cromyophthoron* were grown in dextrose solution and onion decoction in which a constant pH value was maintained with occasional introductions of adjusting reagents, such as 0. 2 N HCL and KOH.

Inorganic salts were used for the preparation of a 2 per cent dextrose solution in the following proportions.

Distilled water	1000 cc.
$MgSo_4$	2.12 grams
$Ca(NO_3)^2$	0.71 do
KH_2PO_4	1.36 do
$Fe(NO_3)_3$, 5% solution	1 cc.

An onion decoction also was prepared by cutting 200 gms. of peeled onions into small pieces and boiling them in 500 cc. of water that had been acidified previously with 25 cc. of 5N HCL. The mixture was boiled for half an hour, then filtered through cotton and the filtrate treated with 15 cc. of 5N NaOH.

Portions of 500 cc. of these solutions were placed in 1-liter Erlenmeyer flasks and adjusted to different hydrogen-ion concentrations before their

TABLE 1.—*Volume (cc.) of reagent (0.2N KOH) required per liter to adjust H-ion concentrations between pH values of 4.0 and 8.0*

pH	4.0	4.5	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0
4.0	.....	0.4	0.8	1.0	1.8	2.6	5.0	6.0	7.6	9.2	11.5	14.5	17.5	21.7	25.9	30.8	33.7	36.5
4.5	.....	.....	0.4	0.6	1.4	2.2	4.6	5.6	7.2	8.8	11.1	14.1	17.1	21.3	25.5	30.4	33.3	36.1
5.0	.....	.....	.....	0.3	1.0	1.8	4.2	5.2	6.8	8.4	10.7	13.7	16.7	21.0	25.1	30.0	32.9	35.7
5.2	.....	.....	.....	.....	0.8	1.6	4.0	5.0	6.6	6.2	10.5	13.5	16.5	20.7	24.9	29.8	32.7	35.5
5.4	.....	.....	.....	.....	.....	0.8	3.2	4.2	5.8	7.4	9.2	12.7	15.7	20.0	24.1	29.0	31.9	34.7
5.6	.....	.....	.....	.....	.....	.....	2.4	3.4	5.0	6.6	8.9	11.9	14.9	19.1	23.3	28.2	31.1	33.9
5.8	.....	.....	.....	.....	.....	.....	.....	1.0	2.6	4.2	6.5	9.5	12.5	16.7	20.9	26.8	29.7	32.5
6.0	.....	.....	.....	.....	.....	.....	.....	.....	1.6	3.2	5.5	8.5	11.5	15.7	19.9	25.8	28.7	31.5
6.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.6	3.9	6.9	9.9	14.1	18.3	24.2	27.1	29.9
6.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.3	5.3	8.3	12.5	16.7	22.6	25.5	28.3
6.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3.0	6.0	10.2	14.4	20.3	23.2	26.0
6.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3.0	7.2	11.4	18.3	21.2	24.0
7.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.8	8.4	16.3	19.2	22.0
7.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.2	12.1	15.0	17.8
7.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7.9	10.8	13.6
7.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6.5	9.0
7.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6.5
8.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

inoculation with *F. cromyophthoron*. Solutions of 0.2N KOH and H_2SO_4 were used for adjusting the reaction of the culture media. Table 1 was prepared to facilitate the work. The volume of KOH indicated as necessary for shifting the reaction of the medium from a higher to a lower acidity was determined on sterile dextrose media composed as mentioned above. With inoculated media all the values have to be changed slightly to overcome the interference of the various colloidal substances released in the solution by the organism. This interference may be approximately determined after brief experience with the organism.

The apparatus described by the writer in another paper (31) was used for the growth of the organism under sterile conditions and at adjusted hydrogen-ion concentrations. The adjusting reagent was added to the culture medium after a determination had been made of its hydrogen-ion concentration. Such adjustments of the pH value were made daily, this having proved very satisfactory and practical. The volume of the culture medium withdrawn from the flask for a single determination of pH averaged between 5 and 10 cc.

Changes in pH Produced by Fusarium Cromyophthoron on Media of Different Initial pH

The organism was grown in both types of media mentioned previously at different hydrogen-ion concentrations. The changes produced in these media as the result of the metabolic activities of the organism were recorded as closely as possible. Tables 2 and 3 contain the information on the utili-

TABLE 2.—*Changes in the pH of dextrose culture solution produced by Fusarium cromyophthoron together with tests indicating presence or absence of free sugars.* (See figure 1.)

Date, 1923	Changes produced by *Fusarium cromyophthoron* in cultures with different initial pH											
	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar
Apr. 10	3.0	+	4.0	+	5.0	+	6.0	+	7.0	+	8.0	+
13	3.2	+	4.0	+	5.3	+	5.8	+	6.8	+	7.8	+
15	4.0	+	4.2	+	5.2	+	5.3	+	6.4	+	7.4	+
18	3.6	+	3.7	+	5.0	+	5.1	+	6.2	+	7.2	+
20	3.6	+	3.7	+	4.5	+	4.0	+	6.0	+	6.2	+
23	3.8	+	3.8	+	4.0	+	3.8	+	5.3	+	5.9	+
25	3.8	+	3.8	+	3.8	+	4.1	+	5.0	+	5.6	+
30	3.8	+	4.6	−	3.8	+	5.2	−	4.8	+	5.6	+
May 4	4.2	+	4.8	−	4.0	+	5.6	−	4.6	+	5.5	+
9	4.6	−	5.2	−	4.3	−	5.8	−	.....	−	.....	−

TABLE 3.—*Changes in pH of onion decoction medium produced by Fusarium cromyophthoron together with tests indicating presence or absence of free sugars*

Date, 1923	Changes produced in the initial pH by the fungus											
	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar
Apr. 10	3.8	+	4.2	+	4.6	+	5.7	+	6.0	+	7.0	+
13	4.0	+	4.6	+	4.6	+	5.5	+	6.0	+	7.0	+
16	3.9	+	4.2	+	5.0	+	5.5	+	6.0	+	7.0	+
18	4.0	+	4.2	+	5.0	+	5.5	+	6.0	+	6.8	+
20	4.1	+	4.3	+	5.0	+	5.3	+	5.8	+	6.8	+
23	4.2	−	4.4	+	5.0	+	5.3	+	5.8	+	6.4	+
25	4.2	−	4.5	−	5.2	−	5.4	−	5.6	+	6.0	+

zation of the sugar content of the solution by the organism and also changes in pH values. Figure 1 presents the changes in pH graphically. Tables 4 and 5 and figure 2 indicate the volume of each reagent required in the different cases to maintain constant pH values.

TABLE 4.—*Volume of adjusting reagents, in cc., required to neutralize the changes produced in the initial H-ion concentration of dextrose solutions of different pH by Fusarium cromyophthoron*

Date, 1923	pH determinations and volumes of reagent required for adjusting pH											
	pH	HCL	pH	KOH	pH	KOH	pH	KOH	pH	KOH	pH	KOH
Apr. 10	3.0	0	4.0	0	5.0	0	6.0	0	7.0	0	8.0	0
13	3.2	1	3.8	1	4.8	2	5.8	3	6.8	2	7.8	4
15	3.1	1	4.0	1	4.7	4	5.8	5	7.0	5	7.7	9
18	3.1	1	4.0	1	4.8	5	5.8	7	6.8	7	7.7	16
20	3.0	1	3.8	2	4.8	6	5.9	8	6.7	9	7.6	18
23	3.1	1	3.8	2	4.8	7	5.8	8	6.8	10	7.6	20
25	3.0	1	4.0	1	4.9	3	6.0	6	6.7	10	7.6	20
27	3.0	2	4.0	1	4.9	1	6.0	4	6.7	10	7.7	20
30	3.0	2	4.0	0	5.4	0	6.0	0	6.8	12	7.8	25
May 4	3.2	2	4.3	0	5.8	0	6.0	0	6.8	12	7.7	25
9	3.2	2	4.6	0	5.8	0	6.0	0	6.8	12	7.7	20
11	3.2	2	4.7	0	6.0	0	6.0	0	6.8	10	7.8	15
29	5.6	0	6.7	0	7.2	0	7.4	0	7.4	0	8.4	0
Total		16		9		28		41		99		192

The results obtained indicate that *F. cromyophthoron* is capable of changing the initial reaction of its substratum by the liberation of either acidic or basic substances, this depending on the initial reaction of the solution. The magnitude of the produced changes may be measured by

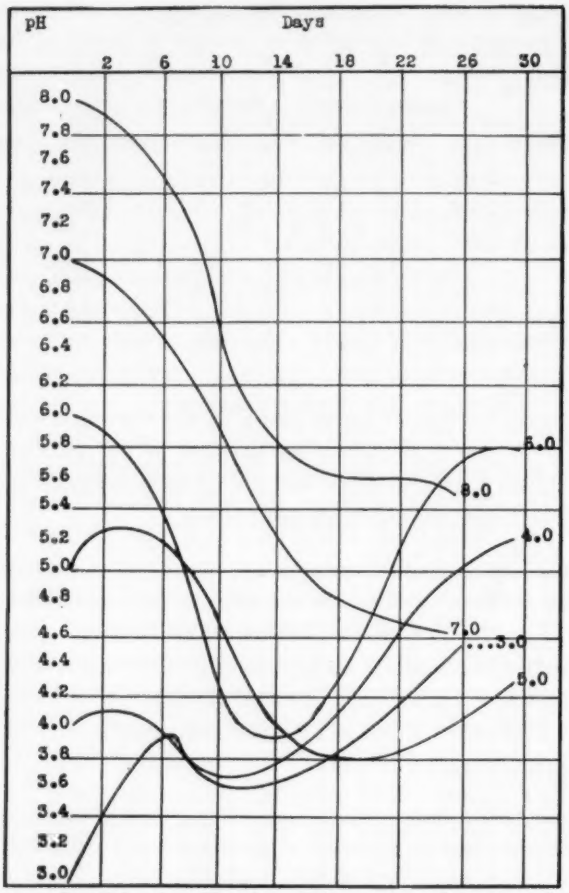


FIG. 1. Changes produced in the pH value of the various dextrose culture solutions by *F. cromyophthoron*. (See table 2.)

TABLE 5.—*Volume of adjusting reagents, in cc., required to neutralize the changes produced in the initial pH of onion decoction by Fusarium cromyophthoron*

Date, 1923	pH determinations and volumes of reagents required for adjusting pH											
	pH	HCL	pH	KOH	pH	KOH	pH	KOH	pH	KOH	pH	KOH
Apr. 10	3.0	0	4.0	0	5.0	0	6.0	0	7.0	0	8.0	0
13	3.0	1	4.3	1	5.1	1	6.0	2	6.8	3	7.7	15
16	3.2	2	4.0	1	5.0	0	5.8	3	6.8	6	7.8	25
18	3.1	2	4.1	2	5.0	0	5.8	4	6.8	8	7.7	30
20	3.0	2	4.0	2	5.0	0	6.0	5	6.7	10	7.8	30
23	3.1	3	4.2	2	5.0	0	5.7	4	6.7	13	7.7	35
25	3.0	3	4.0	1	5.0	0	5.8	4	6.8	15	7.7	35
30	3.0	2	4.0	0	5.0	0	6.0	0	7.0	0	7.9	30
May 30	5.8	0	6.2	0	6.8	0	7.4	0	7.6	0	8.6	0
Total		16		9		1		22		55		200

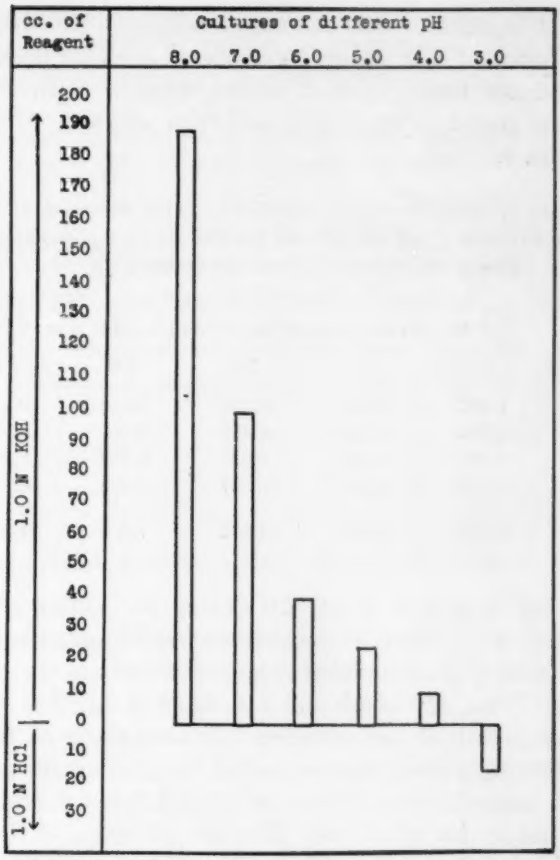


FIG. 2. Volume of 0.2 N KOH and HCl required to maintain the initial pH of dextrose solutions inoculated with *F. cromyophthoron*.

the volume of the reagent used in every case. Tables 2 and 3 indicate that the formation of acids may cease and alkalinity may develop in media after all the sugar has been consumed by the organism.

Vegetative Growth of Fusarium Cromyophthoron at Different pH Values

A number of 1-liter Erlenmeyer flasks equipped with the necessary accessories for controlling pH under aseptic conditions (31) were used for the growth of the fungus. To each flask 500 cc. of the dextrose solution were added, and the pH values were adjusted in each case previous to inoculation with the fungus. Four different series were employed labeled A, B, C, and D, and representing growth periods of 7, 15, 22, and 29 days. All inoculations were made the same day, using as nearly as possible, the

same amount of inoculum. The amount of mycelium produced at the end of the growth period of each series was recovered from the flask by filtration through a hardened filter paper of known weight. Both mycelium and paper were dehydrated in every case and then weighed. The results are recorded in table 6.

TABLE 6.—*Grams of dried mycelium produced in onion decoction at adjusted H-ion concentrations of pH 3.0, 4.0, 5.0, 6.0, and 7.5 by 7-, 15-, 22- and 29-day-old cultures of Fusarium cromyophthoron*

Series	Age in days	Weight in grams of mycelium in cultures of different pH					
		3.0	4.0	5.0	6.0	7.0	7.5
A	7	0.073	0.175	0.230	0.240	0.170	0.150
B	15	0.193	0.454	0.478	0.504	0.500	0.485
C	22	0.188	0.302	0.387	0.400	0.252	0.355
D	29	0.278	0.288	0.307	0.380	0.325	0.327
Total		0.732	1.219	1.402	1.524	1.247	1.317

The organism grew best at pH 6.0 during the 15-day growth period. This indicates that the arrested development of the organism beyond the fifteenth day is due to the depleted supply of sugars in the culture media and also to autolysis. Autolysis did not occur at pH 3.0, where also no decrease in the growth of the organism had been observed throughout all four series. Fehling's-solution tests proved that the organism had utilized completely the sugars in the cultures of pH 4.0, 5.0, 6.0, and 7.5 of series C and D, but not in that of pH 3.0. The low pH value of the last culture may account for the slower utilization of sugars and inhibition of autolysis. It seems that autolysis is inevitable after the organism has used up the total food substances of the culture media. The rise of the pH value of the solution during autolysis is due to the formation of ammonia resulting from the breaking down of the protoplasmic proteins of the dead fungus hyphae.

The Effect of Temperature on Vegetative Growth

The dextrose solution mentioned was used with 2 per cent agar for the preparation of a solid medium. The mixture was adjusted to different H-ion concentrations before sterilization. Duplicate samples were used to determine any changes in the pH value after sterilization. For the growth studies of this fungus petri-dish cultures were found very suitable. About 15 cc. of the medium were poured in a petri dish and after solidification a few spores of the fungus from a sporodochium were planted in the center. Five petri dishes were used for each pH value and each temperature. The

growth of the organism was determined by measuring the diameter of the colony at intervals of 48, 70, 96, 120, and 144 hours. The results obtained from these measurements are recorded in table 7.

TABLE 7.—*Diameter growth in mm. of colonies of Fusarium cromyophthoron on dextrose agar of different H-ion concentrations at 20° and 28° C.*

Age of culture, hours	Temperature, degrees C.	Growth in mm. of colonies in cultures of different initial pH				
		4.0	5.0	6.0	7.0	8.0
48	20	9	10	10	10	9
70	20	19	20	20	21	17
96	20	28	28	28	30	25
120	20	38	39	40	41	37
144	20	50	51	51	53	50
70	28	34	34	33	32	32
96	28	41	44	46	43	41
120	28	57	59	61	60	58
144	28	70	74	75	76	75

The results indicate that temperature alone, independent of the H-ion concentration of the culture medium, influences appreciably the vegetative growth of the organism. Higher temperatures, such as 28° C., favor a much more rapid growth than lower temperatures. The influence of the H-ion concentration of the culture medium on the growth of the organism was rather negligible at both higher and lower temperatures. The ineffectiveness of H-ion concentration in solid media may be attributed to the extremely slow movement of such ions to places where a decrease of their concentration has taken place by ions of opposite electric charge.

Discussion of Results

The results obtained on the behavior of *F. cromyophthoron* in culture media of different H-ion concentrations indicate that different concentrations of H-ions influence the metabolic processes and development of the organism. Temperature may, in an analogous manner, stimulate or retard the development of this organism. The changes produced in the pH reaction of the culture solution may be attributed to the formation of acidic or basic metabolic by-products by the fungus. Whether such acids are released by the organism or formed in the solution as the result of sugar assimilation is not known. If the latter is true it is to be assumed that the sugar molecule is oxidized into acids by the enzymes of the organism before assimilation. Such a process, if possible, will contribute to an accumulation of

acids, and in turn to an increase in the acidity of the culture solution. Acids may not be the only substances formed during metabolism. Basic substances may also be formed by analogous processes. The results presented in table 2 at pH 3.0 and in table 3 at pH 3.0 and 4.0 support this contention. The writer (36) has observed that acidic substances may be formed during metabolic activities at pH values above that of the isometabolic point of the substance concerned, and basic substances may be formed below this point. Weimer and Harter's (43) findings on *F. acuminatum*, Wolf's (45) on *Bacillus carotovorus*, Harter and Weimer's (9 and 10) on *Rhizopus tritici*, and Paine and Chaudhuri's (25) on *Bacillus solanisaprus* prove beyond any doubt that organisms are more apt to produce acid reaction during the assimilation of carbohydrates, and basic reaction when proteins are utilized. The writer (36) has found that if peptone alone is used in media there is no acidic reaction.

Young and Bennett (46), MacInnes (24), Karrer (19), and Hopkins (15) have found that alkalinity may occasionally be produced by various organisms. Such an alkalinity, in the cases cited, may be due to ammonia resulting from the hydrolysis of peptone, or protein of the media, or from autolysis.

In regard to the influence of temperature on the development of various species of *Fusarium*, different investigators have found that not only the development of these organisms is influenced by temperature, but their pathogenicity also. Link (20), Clayton (4), Goss (7), Jones and Tisdale (18), and Tisdale (39) have found those temperatures which favor a rapid development of Fusaria favor, in a similar manner, the development of rots and wilts. Bisby (2) has found that Fusaria grow best at temperatures between 20° and 25° C. As different species, however, vary considerably in their thermal response, Bisby's statement does not apply throughout the entire group of species of the genus *Fusarium*.

PHYSIOLOGICAL BEHAVIOR OF ALLIUM CEPA

The following physiological studies were conducted for the purpose of obtaining some information on the behavior of the normal onion plant, this being essential for comparative studies under pathological conditions.

CULTURAL METHODS

Onion bulblets and seeds were germinated on sand and allowed to grow for 15 days, until they had produced a growth of 10–20 cms. in both tops and roots, and then were transferred to stoneware jars of approximately 8 liters capacity. The seedlings were suspended, in the case of water cultures, through a 12 × 12 × 1-inch wooden board into which had been bored 10 holes, ¾ inch in diameter. Only one seedling was inserted in each hole, making the total number in each crock 10.

The culture solution used for the growth of the seedlings consisted of 2.12 gms. Mg SO_4, 0.71 gms. Ca $(NO_3)_2$, 1.33 gms. KH_2PO_4 and 1 cc. of a 5 per cent $Fe(NO_3)_3$ solution per liter. Different portions of this solution were adjusted to different H-ion concentrations by the addition of 0.2 N, H_2SO_4, and KOH. The soil cultures were prepared by mixing equal volumes of Oakley sand and peat. Different volumes of 3 N of H_2SO_4 or KOH were added to produce the desired H-ion concentration in the different soil cultures.

BEHAVIOR OF ALLIUM CEPA AT DIFFERENT H-ION CONCENTRATIONS

The behavior of onion plants at different H-ion concentrations was carefully observed and recorded. The effect of the pH on the plants was studied in two different ways: In the first case the changes that had been produced in the H-ion concentration of the culture solution by the growth of the plants were not adjusted, but in the second all such changes were adjusted by the addition of various volumes of 1 N H_2SO_4 or KOH. Determinations of the pH of the different solutions were made at rather frequent intervals, and any changes that had resulted in the second case were properly attended to. The results obtained by growing plants in cultures of different pH are recorded in tables 8 and 9.

TABLE 8.—*Changes in pH produced by Allium cepa growing in culture solutions with different initial H-ion concentrations*

Date, 1922	Changes in pH of the ten different cultures									
	1	2	3	4	5	6	7	8	9	10
May 22	4.0	5.3	5.3	5.5	5.5	6.0	6.0	6.5	6.5	8.0
25	4.2	5.4	5.4	5.6	5.5	6.0	6.0	6.4	6.4	—
29	4.3	5.5	5.7	6.0	5.6	6.0	6.0	6.4	6.4	—
31	4.4	5.7	5.9	6.0	5.8	6.0	6.0	6.5	6.6	—
June 1	4.8	5.8	5.9	6.0	5.8	6.2	6.2	6.5	6.7	—
4	5.1	6.0	6.0	6.0	6.0	6.2	6.3	6.6	6.8	—
6	5.5	6.0	6.0	6.3	6.0	6.4	6.0	6.7	6.7	—
9	5.4	6.0	6.0	6.5	6.0	6.2	6.0	6.7	6.6	8.0
15	5.4	6.0	6.0	6.6	6.0	6.2	6.6	6.6	6.3	7.5
19	5.2	6.0	6.0	6.7	6.0	6.5	6.6	6.8	6.8	7.5
22	5.3	6.0	6.0	6.7	6.2	6.6	6.6	6.8	6.8	7.4
24	5.3	6.0	6.0	6.7	6.3	6.6	6.6	6.9	6.8	7.4
27	5.3	6.0	6.0	6.7	6.5	6.6	6.6	6.8	6.8	7.3
30	5.4	6.0	6.0	6.7	6.5	6.5	6.6	6.7	6.7	7.3
July 1	5.3	6.0	6.0	6.7	6.5	6.5	6.5	6.6	6.5	7.2
4	5.3	6.0	6.0	6.6	6.4	6.5	6.6	6.6	6.6	7.0

TABLE 9.—*Volume, in cc., of normal HCL or KOH required to neutralize the changes produced in the pH of the culture solution by Allium cepa, and readings of the prevailing atmospheric temperature during day and night*

Date, 1923	Temperature, degrees F.		pH of cultures and volume of reagents used to adjust pH																	
	Day	Night	pH	HCL	pH	HCL	pH	HCL	pH	HCL	pH	HCL	pH	reagent	pH	KOH	pH	KOH	pH	KOH
Jan. 4	68	55	4.0		4.5		5.0		5.5		6.0		6.5		7.0		7.5		8.0	
9	72	52	4.0		4.5		5.0		5.5		6.0		6.5		7.0	8.0	7.3	10.00	8.0	
15	70	50	4.0		4.5		5.0		5.5		6.2		6.5		7.1		7.5		7.7	20.0
22	60	50	4.4	0.5	5.0	0.5	5.1		5.5		6.2		6.5		7.0		7.5		8.0	
25	80	45	4.3	0.5	4.8	0.5	5.3	0.5	5.5		6.1	0.7	6.5		7.2	2.0	7.5		7.9	
27	60	45	4.4	1.0	5.0	1.0	5.2	0.5	5.5	0.5	6.1	0.3	6.5		7.2	5.0	7.4	5.0	7.8	10.0
30	75	45	4.2	1.0	4.7	0.5	5.0	1.0	5.5	0.5	6.1	0.5	6.5		6.8		7.5		8.0	
Feb. 2	70	45	4.2	2.0	4.7	0.5	5.0	0.5	5.5		6.1	0.5	6.5		7.0		7.5		8.0	
6	70	45	4.2	3.0	5.0	2.0	5.2	1.5	5.5	0.5	6.1	0.3	6.5		7.0		7.3	5.0	7.8	10.0
9	82	48	4.2	2.0	4.5		5.0		5.6	1.0	6.1	0.2	6.5		7.0		7.5		8.0	
13	85	50	4.0	1.0	5.0	2.0	5.0	1.0	5.6	1.0	6.0		6.5		7.0		7.4	5.0	7.7	10.0
15	90	55	4.5	5.0	4.7	1.0	5.2	1.5	5.5	0.2	6.1	0.5	6.5		7.0	1.0	7.5	1.0	7.9	5.0
16	88	52	4.0		4.3		5.0	0.5	5.5	0.3	6.0		6.5		7.0	1.0	7.5	1.0	8.1	
19	80	55	4.0		4.8	2.0	5.0	1.0	5.5	0.5	6.0		6.5		7.0	1.0	7.4	4.0	8.0	
22	80	55	4.0		4.5		5.0		5.5		6.0		6.5		7.0		7.5			
24	85	55	4.5	4.0	4.7	2.0	5.2	1.5	5.7	2.0	6.0		6.5		6.9	2.0	7.3	5.0	7.6	15.0
Mar. 2	75	50	4.0		4.5		5.0		5.5		6.0		6.5		7.0		7.4	4.0	7.8	10.0
Total reagent				20.0		12.0		9.5		6.5		3.0		None		20.0		40.0		80.0

The results obtained, on the behavior of onion seedlings in culture solutions of different hydrogen-ion concentrations, indicate that the plants increased the pH in certain cultures and decreased it in others.

The changes were more pronounced at the two extremes, namely, at pH 4.0 and 8.0, and less in the cultures of intermediate pH reactions. The magnitude of the changes produced may be better understood by comparing the total volumes of the reagent used for adjusting the reaction of the solution (Table 9 and Fig. 3). There were no changes at pH 6.5 or, if any, they were very slight and difficult to detect. The changes in the cultures of higher or lower pH value were, however, quite appreciable.

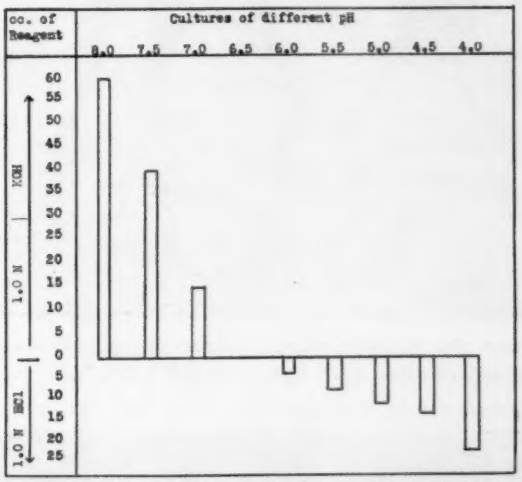


FIG. 3. Volume of 1.0 N KOH and HCl required to maintain the initial pH value of the various culture solutions in which *Allium cepa* was growing.

TABLE 10.—*Dry weights in grams of lots of 10 plants of Allium cepa growing in culture solutions*

Series	Date, 1922	Weight in grams of plants growing in culture solutions of different pH							
		4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5
A	May 22 to July 4	20.0	21.0	28.5	29.8	25.0	20.0	14.0	5.0
B[a]	Jan. 4 to Mar. 2	13.0	16.0	26.0	29.0	28.5	34.5	30.0	18.0

[a] Series B consists of the cultures reported on in table 9.

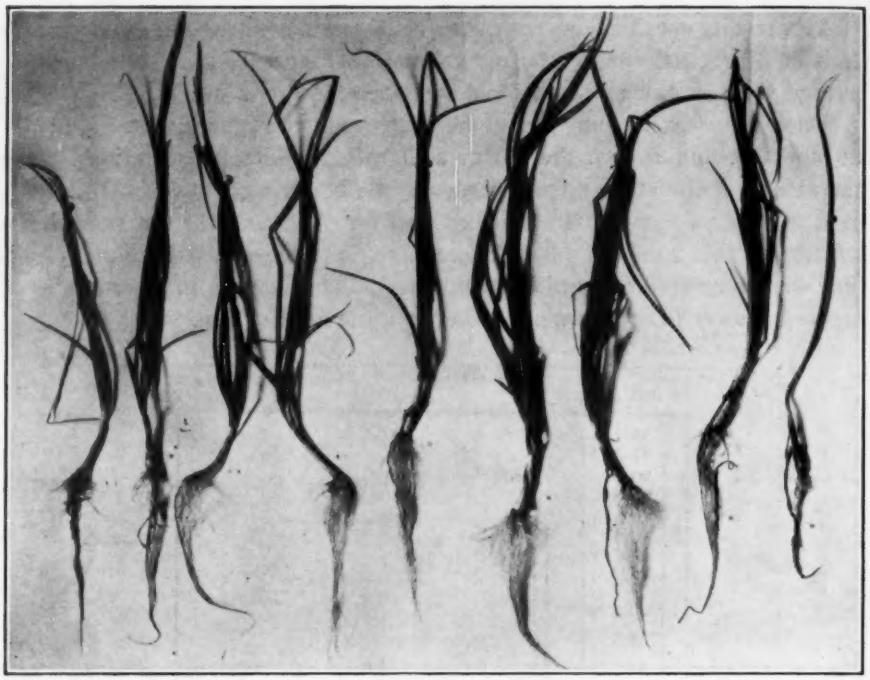


FIG. 4. Entire onion plants, one from each culture, representative of the average type. From left to right: pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0.

FIG. 5. The root system of onion plants, one from each culture, representative of the type. Cultures from left to right: pH 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 7.5, and 8.0.

The development of the root system and tops of the different plants seems to have been influenced considerably by the initial pH value of the culture solution (Figs. 4 and 5). This is shown in table 10 by the weights of the plants. These plants, 10 in each group, were dehydrated at 100° C. and their dry weight determined. The plants of series A were grown during the summer and those of series B during the winter months.

The different values obtained for the weight of the plants of the different cultures indicate that the H-ion concentration of the solution is responsible to a great extent for the differences in the growth of these plants. There is, however, a certain disagreement in the growth obtained in Series A and B at the same H-ion concentrations and the same culture solution. It seems that this difference is climatic, having to do more with sunlight and heat rather than with any other single factor, inasmuch as the plants were grown during the two different seasons. The results indicate that the optimum pH range for the growth of onions is between 5.5 and 6.5.

Growth of Onions in Soil Cultures of Different pH Values

The soil substratum was prepared, as mentioned before, with equal volumes of peat and Oakley sand and adjusted to different hydrogen-ion concentrations by means of 3 N H_2SO_4 or KOH. The H-ion concentration of the different cultures was determined on a Hildebrand electrode (12). The seedlings, germinated on sand, were as uniform as possible at the time of their transfer to the different cultures. The plants were allowed to grow from February 5 to May 25, 1922. The number of plants employed in each culture was 25, 5 plants to each 2-gallon stoneware jar. The results obtained are recorded in table 11.

TABLE 11.—*Weight, in grams, of green onion plants grown in soil cultures of different initial pH from February to May and at temperatures fluctuating between 50° and 110° F.*

Data taken	Series					
	1	2	3	4	5	6
Initial pH	3.6	5.0	6.0	6.8	7.6	8.0
Final pH	4.0	5.5	6.2	7.0	7.4	7.6
Weight in grams	500	1000	1300	1350	500	250
No. dead plants	10	0	0	0	15	20
No. etiolated plants	10	3	0	1	10	5
No. healthy plants	5	22	25	24	0	0

The behavior of onion plants in soil cultures is comparable to their behavior in culture solutions. The plants in both soil and solution cultures

either did not grow well or showed signs of distress at high acidity or alkalinity. It was particularly noticeable in soil cultures in which the titratable acidity or alkalinity was greater than that of the water cultures; in fact, some of the plants became etiolated and died. The optimum pH value for the growth of these plants was found to be between pH 5.0 and 7.0, this being almost the same as that reported for solution cultures. The final pH value of the different cultures varied somewhat from that of the original. There was an increase in pH value in those cultures that had an initial pH below 7.0, and a decrease in those whose initial pH was above 7.0.

Discussion of Results

The general belief, with respect to changes produced by plants in the reaction of their substratum, is that they tend to bring the final reaction nearer to the neutral point by the liberation of either acids or bases, as the case may be. Such conclusions have been based on observations made on culture solutions of more or less identical composition. With culture solutions of somewhat different composition it is possible that the same plant may behave differently.

The studies of Hoagland and Davis (14) on this subject are very enlightening. These investigators found that the H-ion concentration of plants transferred from a complete culture solution of pH 5.0 to a dilute solution of K_2SO_4, as well as to solutions of a number of other salts, increased from pH 5.0 to 3.2. They have suggested a hypothesis by which such phenomena may be intelligently explained. They state that the complexity of factors which influence the mechanism of absorption of the different ions from the solution is responsible for many of the changes of the initial H-ion concentration of the different cultures. They cite a number of examples which tend to emphasize the belief that under acid conditions anions are absorbed from the solution at a greater rate than the corresponding cations, providing that both are of equal valence. Under alkaline conditions the reverse reaction takes place. Bicarbonate ions (HCO_3) are considered to play the greatest rôle in the change of the initial H-ion concentration of the culture solution in either direction; *e.g.*, toward alkalinity or acidity. Under acidic conditions bicarbonate ions may replace the absorbed NO_3-ions, Cl-ions, etc., and form salts of a basic nature. Under alkaline conditions they may influence with their acidic properties the initial H-ion concentration of the culture solution. Theron's studies (38) on the influence of plant absorption on the initial pH reaction of the substratum bring out the same evidence as discussed above by Hoagland and Davis. Although Theron observed that wheat plants produced the best growth at pH 5.5, yet the culture which suffered least from changes in the reaction

had a pH value of 6.8. Pantanelli's (26) contention is that plants tend to maintain a favorable H-ion concentration by modifying objectionable reactions for best growth. Hoagland's (13) findings on barley strengthen the evidence, so often presented by many investigators, that different plants behave differently at different H-ion concentrations, barley thriving best at pH 7.0, whereas wheat thrives at 5.5. If Robbins' (28) interesting investigations on the isoelectric point of plant tissues establish definitely that plant tissues behave as ampholytes, then we may be able to understand better the type of reaction that takes place between the plant tissues, on the one hand, and the culture solution on the other.

There is no doubt that reactions of this type are responsible for the changes resulting in the initial H-ion concentration of the culture solution. The investigations of the writer (36) on the behavior of *Fusarium cromyophthoron* at different H-ion concentrations in culture media of carbohydrates, glucosides, and proteins, have shown that the changes that were produced in the pH value of the cultures above the isometabolic point of the particular substance were due to the liberation of organic acids. The changes, however, that had been produced in the reaction of the cultures of a lower pH value than the isometabolic point of the substance under consideration were due to the generation of HCO_3 with young cultures, and to NH_4OH with old cultures. Results obtained with fungi cannot be compared to those obtained with green plants owing to the great diversity in the nutritional requirements of the two groups of organisms.

The better growth produced by onion plants during the summer months in more acid cultures, as compared with that during the winter months, may be due to the greater photosynthetic activities of the plants, on the one hand, and to the availability of the different salts on the other. There is no doubt that plants are producing more organic matter during the summer than the winter months and that the availability of the majority of elements at more acid reactions is greater than at less acid or alkaline reactions, in spite of the fact that no precipitate of insoluble salts is apparent and the plants are grown in water cultures. High H-ion concentrations may, besides increasing the solubility of the various salts, hasten the escape of CO_2 accumulations in the culture solution, which, in turn, helps the diffusion of atmospheric oxygen and also prevents the precipitation of calcium. Higher acidities during the summer months—the busiest period for plants—may be more favorable for the development of plants. The question then arises: "Are we permitted to consider that there is only one pH value for the best development of plants during both winter and summer months?" The answer to this is no!

The changes in the initial H-ion concentration of the different soil cultures were possibly not produced by the plants grown therein, but by the various acidic and basic substances of the soil. The results indicate that

those cultures with an initial pH value lower than 7.0 had a higher final value, and those with a higher initial value had a lower final pH. Gimingham (6) found that similar changes were produced in soils left in contact with water and then exposed to air for some time without green plants. He did not attempt to exclude microorganisms.

THE INTERRELATIONS OF FUSARIUM CROMYOPHTHORON AND ALLIUM CEPA IN THE DEVELOPMENT OF THE PINK-ROOT DISEASE

Symptoms of the Disease

Roots are the plant organs that are more frequently invaded and killed by the causal organism (Fig. 6). A pink pigment is formed within the invaded tissues; hence the name pink root. The invaded roots die in the majority of cases. The cortical tissue undergoes decomposition leaving behind the exodermis and stele of the root. The pink pigment may be detected on the dried roots of onions sold in the market as well as on those of onions in the field. The pigment in dried roots is not of an intense color, but it can be brought out merely by wetting the tissues.

Nature of the Pink Pigment

As the development of pigment in diseased roots was not well understood, certain experiments were carried out to furnish the necessary information. Onion scales, some of which were taken from the center and others from the periphery of the bulb, and roots were subjected to various treatments of acids and bases with the object of forcing out the manifestation of their pigments. The reagents were heated in every case to 80° C., and the tissues then immersed in the solution for two minutes. The results obtained from these treatments are recorded in table 12.

TABLE 12.—*Influence of acids and bases on the development of pigment in onion scales and roots, when exposed to indirect sunlight before treatment and when treated immediately after detachment from the bulb*

Reagent and concentration	Hours of exposure to light	Presence of pigment on	
		Scales	Roots
0.1 N HCL	0	−	−
do do	24	+	−
10% Oxalic acid	0	−	−
do do	24	+	−
do Malic acid	0	−	−
do do	24	+	−
do Tartaric acid	0	−	−
do do	24	+	−
do Citric acid	0	−	−
do do	24	+	−
1.0 N NH_4OH	0	−	−
do do	24	−	−

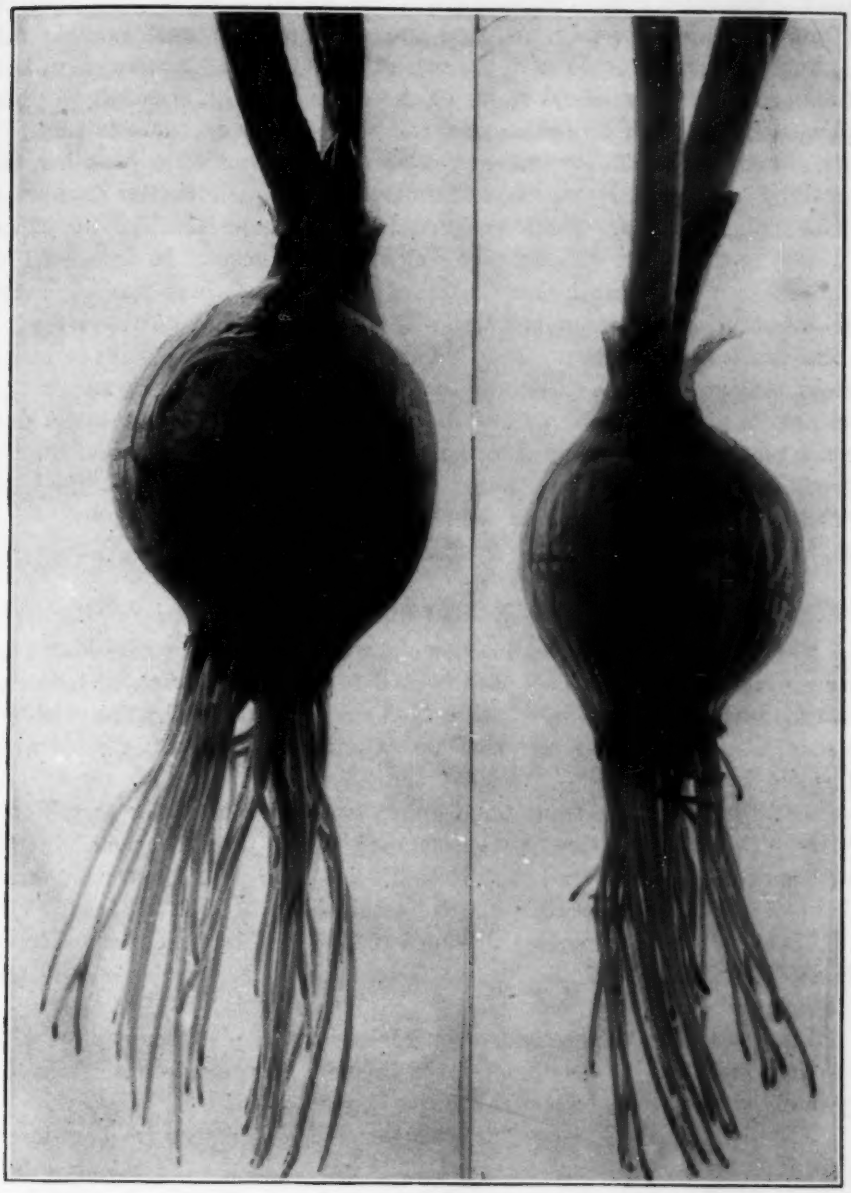


FIG. 6. Left: Plant grown in sterile water and inoculated with *F. cromyophthoron*, showing the initial development of pink root symptoms in the root tips. Right: Plant growing in sterile water and inoculated with *F. cromyophthoron* showing advanced development of pink-root symptoms on root tips.

The tabulated results indicate that acids brought out the pink pigment of onion scales previously wounded and exposed to indirect sunlight for 24 hours, but failed to do so in the case of roots that had received identical treatment. The peripheral scales of the onion yield quite readily a pink pigment if treated with acids alone, and without any exposure to sunlight after wounding. The formation of pigment in lily bulbs has been investigated by Mirande (22), who says that oxidation and acidification take place before pigmentation. He has demonstrated a strict localization of an oxidase in those cells which produce anthocyan pigment. In scales of lily bulbs, which were detached and exposed to the light, there was a general acidification due to wounding and a special acidification correlative with pigmentation. The negative results obtained by the action of acids on onion roots indicate that these organs lack either the oxidase enzyme or the anthocyan pigment or both. The development of the pink pigment of diseased roots may be attributed to the parasitic organism. Fusarium species produce various pigments on artificial as well as on natural media, the hues of which may vary with changes in the pH value of the surrounding solution (33).

Inoculation Studies in Water Cultures

The onion seedlings used for inoculation purposes were germinated in sterile sand. The same method was used for the germination of bulblets. Both seeds and bulblets were disinfected previous to germination with an alcoholic solution of mercuric chloride. Certain lots were disinfected with formaldehyde.

The seedlings were transferred either to water cultures or to soil cultures. The water cultures were composed of the salts mentioned in the first part of this paper and soil of equal volumes of peat and Oakley sand. The soil cultures were sterilized with formaldehyde.

The seedlings that developed from bulblets were, in certain cases, freed from the scales of the bulblet before planting and, in other cases, left undisturbed.

Pure cultures of *Fusarium cromyophthoron* were placed in contact with the root system of the seedlings at the time of their transference from the germinating bed to the water and soil cultures.

The plants in the water cultures were examined at rather frequent intervals and the developments recorded. The plants in the soil cultures were examined only at the termination of the experiment. The results that were obtained with onion seedlings in both water and soil cultures and under different treatments are recorded in tables 13–17. The pink root symptoms are recorded in percentages. These numbers refer to roots in each group of

10 plants. To illustrate: If in a culture of 10 plants, there are 100 roots regardless of the number per plant and if only 3 out of the total of 100 show symptoms of the disease, the results would be recorded as 3 per cent.

TABLE 13.—*Percentage of roots with symptoms of pink-root disease produced on onion plants freed from the bulblet scales at the time of planting and grown in water cultures of different initial H-ion concentrations*

Initial pH of cultures	Appearance of pink root symptoms during growth				Average wt. of roots grams per plant
	Planted Jan. 20	Inoculated Feb. 8	Mar. 8	Mar. 20	
4.0	None	None	15% pink	20% pink	1.0
4.5	do	do	5 do	5 do	1.5
5.0	do	do	2 do	3 do	2.5
5.5	do	do	1 do	1 do	3.0
6.0	do	do	0 do	0 do	3.2
6.5	do	do	0 do	0 do	3.2
7.0	do	do	0 do	0 do	3.0
7.5	do	do	10% yellow	15% yellow	1.5
8.0	do	5% yellow	15 do	20 do	0.75

TABLE 14.—*Percentage of roots with symptoms of pink-root disease produced on onion plants planted with the bulblet scales attached and grown in water cultures of different initial H-ion concentrations*

Initial pH of cultures	Appearance of pink root symptoms during growth				Average wt. of roots grams per plant
	Planted Jan. 20	Inoculated Feb. 8	Mar. 8	Mar. 20	
4.0	None	None	20% pink	30% pink	0.8
4.5	do	do	15 do	25 do	1.2
5.0	do	do	5 do	10 do	2.0
5.5	do	do	3 do	5 do	2.6
6.0	do	do	1 do	1 do	3.0
6.5	do	do	0 do	1 do	2.8
7.0	do	do	0 do	0 do	2.4
7.5	do	5% yellow	15% yellow	25% yellow	1.0
8.0	do	do	25 do	40 do	0.3

The results obtained in the different experiments indicate that at high H-ion concentrations the development of pink root symptoms is more pronounced than at low H-ion concentrations. This does not mean that plants grown at higher pH values than 7.0 made a wonderful growth; on the contrary, they were stunted more by the effect of OH-ion concentrations than

TABLE 15.—*Percentage of roots with initial and final symptoms of pink-root on onions grown in water cultures of different H-ion concentration from May 1 to June 15, 1923, in the greenhouse. Seedlings inoculated in seedbed, or otherwise, as stated*

Culture	Symptoms	Percentage of infected roots in water cultures of different initial H-ion concentration						Inoculation
		pH 4.0	pH 5.0	pH 6.0	pH 6.5	pH 7.0	pH 7.5	
1	Initial	25	25	25	25	25	25	Seed-bed
	Final	75	75	50	50	40	35	
2	Initial	50	50	50	50	50	50	do
	Final	100	75	100	75	70	70	
3	Initial	75	75	75	75	75	75	do
	Final	100	100	100	100	90	90	
4	Initial	0	0	0	0	0	0	Trans-
	Final	15	5	1	0	0	0	planting
Control	Initial	0	0	0	0	0	0	
	Final	0	0	0	0	0	0	

TABLE 16.—*Percentage of roots with symptoms of pink-root disease produced on onion seedlings, after inoculations with pure culture of Fusarium cromyophthoron, grown in water cultures of different initial pH, at temperature 20° to 34° C. Inoculated May 10, 1923*

Culture	Symptoms	Percentage of infected roots in water cultures of different initial H-ion concentration					
		pH 4.0	pH 5.0	pH 6.0	pH 6.5	pH 7.0	pH 7.5
1	May 25	5	1	0	0	0	0
	June 17	15	5	1	0	0	0
2	May 25	7	0	1	0	0	0
	June 17	20	15	3	1	0	0
3	May 25	2	0	2	0	0	0
	June 17	25	25	5	2	0	0

the plants grown at a lower pH. The pink pigment did not develop on roots grown in cultures of high pH value, but a yellow pigment developed instead.

A comparative analysis of the results obtained with different planting material and recorded in tables 13 and 14 indicates that the plants that had been freed from some of their scales developed less pink root than those that

TABLE 17.—*Condition of onion seedlings, grown in water cultures of different initial pH, and inoculated with Fusarium cromyophthoron, at temperatures from 20° to 34° C.*

Condition of plants	Percentage of normal, stunted, chlorotic, and dead onion plants grown in cultures of different initial H-ion concentration					
	pH 4.0	pH 5.0	pH 6.0	pH 6.5	pH 7.0	pH 7.5
Normal	15	95	100	85	30	10
Stunted	80	3	0	10	30	10
Chlorotic	0	0	0	1	25	50
Dead	5	2	0	4	15	30

were planted with the superficial bulblet scales on. The scales apparently furnish an excellent medium for the saprophytic development of the fungus, thus increasing the inoculum and chances of root infection to a considerable degree.

Table 15 contains the results obtained on onion seedlings inoculated in the seedbed long before transferring to cultures of different hydrogen-ion concentration and also those obtained on plants that were inoculated after transplanting. These results indicate that various concentrations of H-ions had no beneficial effect whatever on seedlings inoculated in the seedbed although they had some influence on the development of symptoms on plants inoculated after transplanting. It may be stated, therefore, that plants already infected with the pink-root disease and submitted to treatments of different H-ion concentrations do not seem to show a reduction of pink-root symptoms, unless new roots are produced that may evade the parasite under very favorable conditions. The color of the pigment on the roots is somewhat different in acid and alkaline solutions, being rose pink in the former and purplish pink in the latter. The slight increase of pink-root symptoms on plants in cultures of pH 7.0 and 7.5 (Table 15) presents a condition that has never been observed before by the writer and is rather difficult to explain. The opinion of the writer, however, is that these tissues that showed an increased development of pink-root symptoms may have been diseased at the time the plants were being transplanted, but had not yet, for reasons not well understood, produced the pink coloration.

Tables 16 and 17 give an account of the percentages of roots with symptoms of pink root produced at different H-ion concentrations and the condition of the plants at the same time. The death rate is higher under high pH values, namely, 7.0 and 7.5; whereas, the development of pink-root symptoms is nil. The death of plants under such conditions may be at-

tributed to the toxic effect of high concentrations of OH-ions. The chlorotic condition developed at high pH values may be attributed to the precipitation of ferric-ions. The stunting of plants grown in cultures at pH 4.0, 7.0, and 7.5 may be due, in the first case, to high concentrations of H-ions and, in the last case, to OH-ions, or lack of certain ions. The development of pink symptoms does not necessarily presuppose the death of the infected plant. Pink-root symptoms associated with certain roots may signify the death of those individual roots, but not of the entire plant. The entire plant may die under certain conditions, but the death rate hardly ever exceeds 10–15 per cent of the infected plants. Stunting more commonly results in plants infected with pink-root disease.

Inoculations in Soil Cultures

The soil was prepared of equal volumes of Oakley sand and peat and adjusted to different pH reactions by additions of different amounts of H_2SO_4 and KOH. It was sterilized with an application of about 2 per cent formaldehyde. Although the soil was not completely sterile, it was free from many objectionable organisms. No attempt was made to keep it free from other organisms, such as those contained in the air or the water used for irrigation after planting. The inoculum, *F. cromyophthoron,* was introduced in the soil in the same hole in which the young plant was placed at the time of planting. The growth of these plants extended from February to July, with temperature fluctuations varying between 14° and 34° C.

The plants grew fairly well in all the different soil cultures, except for the slight stunting that developed in some of them. Some deaths were noted in a few cases. At the termination of the growth period the different plants were removed as carefully as possible from the containers to avoid breaking or loss of any of the roots. The roots were segregated in two distinct groups, the primary and secondary (34), and were studied carefully as to the amount of pink-root symptoms, etc. The findings are recorded in tables 18 and 19.

The evidence presented in these tables indicates that the development of pink-root symptoms may or may not be correlated with parasitism. The fact that pink-root symptoms appear, for the most part, on primary roots after the bulbing period of the plant indicates that relationship of the fungus to the root tissues is of a saprophytic nature, the tissues having previously died from natural causes. The association of the fungus with primary roots may then be considered saprophytic in about 75 per cent of the cases. Parasitism should be restricted to cases where the fungus is found in association with apparently healthy secondary roots showing pink symptoms.

TABLE 18.—*Percentages of primary and secondary roots with symptoms of pink root disease on onion seedlings grown in soil cultures of different initial pH, condition of the roots and readings of the initial and final pH of the soil solution*

pH readings of soil cultures		Percentage of infected roots		Condition of roots (percentage dead)	
Initial	Final	Primary	Secondary	Primary	Secondary
3.5	3.8	10	5	100	25
5.0	5.5	100	1	100	2
6.0	5.9	100	0	100	0
7.0	6.8	50	0	100	0
7.5	7.3	3	0	90	10
8.0	7.6	0	0	100	80

TABLE 19.—*Condition of onion seedlings grown in soil cultures of different initial pH and inoculated with a pure culture of Fusarium cromyophthoron, at temperatures 20° to 34° C.*

Condition of plants	Percentage of normal, stunted, chlorotic, and dead onion plants grown in cultures of different H-ion concentration					
Initial pH	3.5	5.0	6.0	7.0	7.5	8.0
Final pH	3.8	5.5	5.9	6.8	7.3	7.6
Normal	10	90	100	85	20	0
Stunted	85	8	0	15	60	40
Chlorotic	0	0	0	0	30	20
Dead	5	2	0	0	20	40

The development of two sets of roots by onion plants (34) complicates considerably the diagnosis of purely parasitic from saprophytic cases. The death of the primary roots is believed to result from certain histological complications. The association of these roots with pink-root symptoms does not represent parasitism in the true sense of the word.

It has been found, as far as the influence of different concentrations of H-ions on plant growth is concerned, that the influence is the same whether soil or water cultures are involved. The influence of the H-ion concentration on the development of pink-root symptoms is likewise the same whether it applies to water or soil cultures.

The few cases of pink root that have been observed in somewhat alkaline soils may be due possibly to the low moisture content of such soils. The fungus is capable, without any doubt, of producing local changes in tissues not bathed with great quantities, or a continuous film of water, which helps

the free movement of the hydrogen of hydroxyl ions of the soil solution to or from an area. Such reactions may be produced by the by-products of metabolism of the fungus which may be acidic or basic in reaction. In alkaline soil cultures of relatively high moisture content the development of pink root has never been observed by the writer.

The Disease in the Seedbed

Soil of the same type as mentioned before was used for the germination of onion seeds. The pH of this soil was 5.2. Two agate pans $25 \times 35 \times 5$ cm. were used for the growth of the plants, one of which was inoculated with *F. cromyophthoron* at the time the seeds were sown and the other left for control.

The plants in both cultures started to germinate on the tenth day. Symptoms of the disease appeared on the roots after the twenty-fifth day in the inoculated culture; many of the plants also wilted and died.

It was noticed that the severity of the disease, as far as the death of individuals was concerned, could be minimized by a lowering of the moisture content of the seedbed. Nearly 100 per cent of the surviving plants showed symptoms of pink root at the end of 70 days. About 80 per cent of the total number of plants died by the end of this period. The results illustrated in figure 7 show the inoculated pan with the smaller number of plants compared with those in the noninfected pans. The indications are that onion

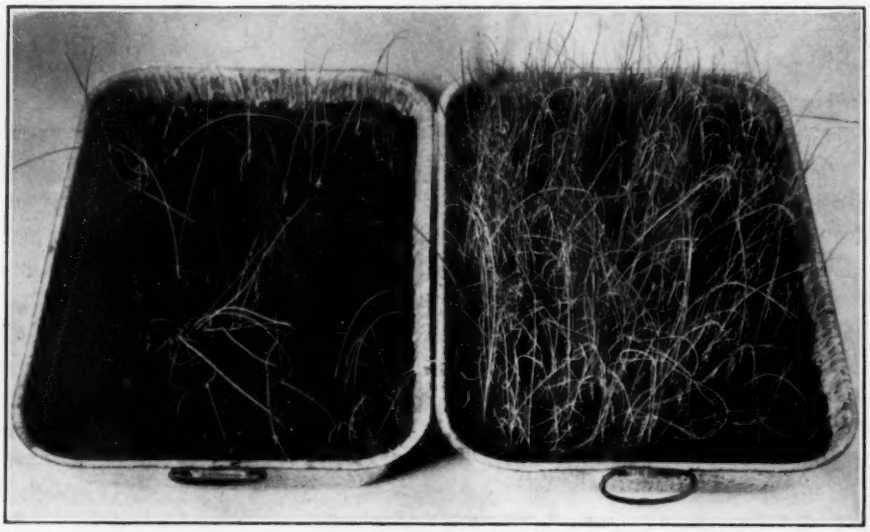


FIG. 7. Left: Onion seedlings inoculated with *F. cromyophthoron* at time of sowing. Right: Onion seedlings not inoculated, but kept for control.

seedlings are very susceptible to pink root, their resistance increasing with age. It is possible to produce almost 100 per cent infection but not death, providing temperature, moisture, and soil acidity are favorable.

DISCUSSION OF RESULTS

Observations made on the development of pink-root symptoms in a number of inoculated onion plants have indicated that the tissue adjacent to the tip of the root is the first to develop pink-root symptoms. Lateral roots may also develop symptoms on the same root area. The pigment, a characteristic symptom of this disease, is the result of the metabolic processes of the fungus on the root tissues. The fungus enters the root of the host plant through the tissues which are least resistant. Such tissues occur near or at the tip, being of embryonic nature and not having undergone differentiation or high specialization. These tissues are neither shielded by the rootcap proper nor protected by such resistant substances as lignin, or suberin. The cementing substance of the cells of the tissues under discussion, being of pectin, is highly susceptible to the hydrolyzing effect of pectinase. Whether the fungus makes its initial invasion into the root by mechanical pressure (3), by means of hydrolytic enzymes, or some other less understood means, it is difficult to say. After the initial invasion the various steps of fungal invasion into the tissues of the host may be easily followed. It usually disintegrates the cortical tissue and all embryonic tissues, leaving intact those tissues that have undergone considerable differentiation. The tissues that are usually left undecomposed on an onion root which shows symptoms of pink root are the exodermis and stele. The pigment is deposited more often on the stele than on the exodermis.

The fungus may invade the tracheids of the root, causing wilt and occasionally death of the young plants. The fungus is not limited in its invasion to the roots alone. It may enter the stem or bulb through the roots, and produce, under proper conditions, disintegration and death of the bulb.

The influence of the H-ion concentration of the solution on the development of pink root deserves consideration in more than one way. The H-ion concentration of the culture solution may influence favorably or unfavorably the development of onion plants as follows: (*1*) H- or OH-ions may either increase or decrease the availability of other ions, and (*2*) high concentrations of either H- or OH-ions may cause injury through their toxic properties. Repeated observations by the writer of the root tips of a number of plants subjected to high concentrations of either H- or OH-ions have shown that such parts of the plant were killed outright. Addoms (1) has also observed that in the roots of wheat plants subjected to H-ion concentrations ranging in acidity between pH 3.47 and 3.94 the protoplasmic contents of the cells were coagulated and flocculated.

In regard to the influence of H-ion concentrations on the growth and parasitic behavior of the fungus, the evidence presented so far, indicates that the fungus is capable of resisting H-ion concentrations as high as pH 3.0 and as low as pH 10.0. Both limits exceed those that onion plants can tolerate. H-ion concentrations ranging between pH 3.5 and 7.5 have no influence whatever on the release of pectinase by the fungus. The writer (35) has found that pectinase may be released by very old and possibly dead hyphae but not by young and healthy hyphae. The fungus therefore may make use of mechanical pressure rather than of pectolysis as an initial step for the invasion of the onion root tissues. Other means, such as the killing of the tissue by toxins, accidental wounds, etc., may open a path to fungal invasion.

Considering temperature as a possible factor for increasing or decreasing pink-root infections, the findings indicate that many more cases of pink-root occur during the warm than in the cool months of the year. Jones and Tisdale (18) have found that higher temperatures increased the rate of infection in flax wilt, and Clayton (4) has reported similar behavior for tomato wilt. The influence of temperature on the development of diseases caused by Fusaria might be of double nature. First, it may stimulate growth in the fungus, which in turn may increase its numbers and also the chances of infection. Second, it may inhibit the normal development of the host, which may result in loss of vitality and susceptibility to infection.

In regard to the occurrence of the pink-root disease in the seedbed, the results are in accordance with those of other investigators working with different species of *Fusarium*. Rose (30) observed, in germination tests of 18 varieties of wheat, that a mortality of from 3 to 98 per cent could be produced by *F. culmorum* var. *leteius* Sher. Hartley (11) found, likewise, that *F. moniliforme* and *F. ventricosum* Ap. et Wr. were pathogenes of the seedbed.

In conclusion, it is the writer's opinion that the presence of pink root symptoms alone on dead roots of the host and the isolation of *F. cromyophthoron* or any other species of *Fusarium* are not sufficient evidence for reliable diagnosis of pathogenicity. The occurrence of pink-root symptoms on apparently healthy or diseased young primary or secondary roots and the presence of the fungus inside the host tissues should be the only reliable criteria for determining pathogenicity.

SUMMARY

The physiological behavior of *Fusarium cromyophthoron* Sid. in cultures of different H-ion concentrations may be summarized as follows:

The growth of this organism is influenced appreciably by the reaction of the culture medium, the total sugar content, and the age of the culture. Best growth was produced at about pH 6.0, although pH values as high as 7.5 and as low as 3.0 did not stop the growth of the fungus.

The initial pH value of the culture solution was changed in the majority of cultures by the metabolic by-products of the organisms, these being acidic and basic in nature. The cultures that suffered the least in their initial pH were those that had a reaction as near as possible to the isometabolic point of the principal nutrient substance of the culture.

The consumption of dextrose was considerably less at pH values lower than 4.0. This is considered to be the isometabolic point for dextrose. Above this pH value the consumption of sugar reached its maximum. Whether the high H-ion concentrations had anything to do with the electric charge of the sugar molecule, or whether a reversal of the electric charge of the proteins of the fungus mycelium had taken place, inhibiting the absorption of sugar molecules at low pH values, the writer is not in a position to state definitely.

After the fungus has absorbed all of the available sugar of the culture medium, its mycelium undergoes autolysis. During this stage the reaction becomes decidedly alkaline owing to ammonia released from the hydrolyzed protoplasmic proteins of the fungus. Autolysis under relatively acid conditions, that is, below pH 4.0, is negligible, or may develop very slowly because of the slow consumption of the sugars of the culture.

Temperature influences vary appreciably the growth of the fungus. A temperature of 28° C. was found more favorable for the growth of this organism than one of 20° C.

The effect of H-ion concentration on the growth of onion plants was found to be as follows:

Onion plants grow best in both water and soil cultures at pH values between 5.5 and 6.5. Higher or lower pH inhibited their growth somewhat.

Onion plants change the initial reaction of their culture solution. The least changed culture was that with an initial pH of 6.5. It is believed that such changes were brought about by the uneven absorption of equivalent ions in the cultures having a reaction above or below pH 6.5. High concentrations of H- or OH-ions were found toxic, the latter being more toxic than the former.

Differences of temperature and sunlight may shift upward or downward the pH scale for the optimum growth of plants. It was found that during the summer months a slightly more acid reaction is more favorable for the growth of onions than in the winter months.

The pathogenicity of *F. cromyophthoron,* measured by the death rate it causes, is higher at pH values above 6.5; whereas, the development of pink-root symptoms is inhibited. The fungus at pH values lower than 6.5 may invade naturally dying roots or may even kill some and produce the typical symptoms of the malady, but it is not capable of wholesale invasion of the roots and death of the plant. At pH values above 6.5, however, the death of roots and plants is more common, although the production of pink-root symptoms is nil.

The pigment characteristic of the pink-root disease is only produced by the fungus at low pH values, namely, between pH 3.5 and 6.0. The invasion of roots is more extensive at a higher pH. The greater death rate of roots grown in cultures between pH 6.5 and 8.0 is believed to be due to the toxicity of high concentrations of OH-ions to the tissues of the host; this condition decreases their resistance considerably to invasion by the fungus.

It is unsafe to draw conclusions on the pathogenicity of *F. cromyophthoron* when associated with the dead tissues of the host. As onion plants produce more than one set of roots, it is possible that the old ones may die of senility, or lose their resistance on account of senility, and become invaded by *F. cromyophthoron* or other facultative parasites. The death of roots under such conditions is not due to the aggressive parasitism of the fungus, but to more than one factor. If, on the other hand, the fungus is found associated with a recently invaded healthy root, then one is justified in associating the death of such a root or roots with *F. cromyophthoron.*

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SOME OBSERVATIONS UPON THE WHITE PINE BLISTER RUST IN NEW YORK

WALTER H. SNELL

In 1923 the writer began some studies of the white pine blister rust in New York for the purpose of obtaining information regarding damage and kindred subjects, for the use of the New York Conservation Department in its campaign of blister rust control. The work was done under the direction of Dr. H. H. York, Forest Pathologist of that Department. Some preliminary data obtained upon the damage caused by blister rust in New York appeared recently (13). The present paper gives the results of certain observations incident to the damage studies.

FUNCTIONS OF THE DIFFERENT SPORE STAGES IN THE LIFE CYCLE OF CRONARTIUM RIBICOLA

When investigation of the blister rust was begun in earnest in 1916, the prevailing idea of the respective parts played by the different spore forms of the fungus was approximately as follows: The aeciospores do little more than initiate the rust by infecting *Ribes* in the spring; the urediniospores formed on *Ribes* are blown a considerable distance and cause new infections, which develop another generation of urediniospores likewise to be blown far and wide, and so on for several generations; and the sporidia perhaps also are able to disseminate the fungus in somewhat extended flights to the pine. The idea was that all these spores disseminated the fungus somewhat widely, with the greater part of the spread accomplished by the uredinial stage because of its repeating character. Indeed, the observations made in scouting for the rust in the southeastern part of the New England States in 1916 left the impression that the disease was spreading very rapidly by means of the long-distance carriage of urediniospores (14, p. 56).

Studies made in the next two years, however, cast doubt upon this idea of the rôles played by the different spore stages, and, although blister-rust workers in general have for some time had a correct idea of the situation, it apparently has not been presented to pathologists in general.

The writer's first intimation of a different conception of the functions of the various spore stages came during the course of some work which he carried out in 1918 near Lewis, Essex County, New York, for the Office of Forest Pathology, United States Department of Agriculture. It was found, for example, that the supposed disseminating stage, the urediniospores, did not disseminate the rust at all, but merely intensified the infection locally within very narrow limits (12, 14). A study of the different spores gave

the clue, and other studies by the writer led to the formulation of a different scheme, presented in manuscript form to the Office of Forest Pathology in 1918. Further work by the writer and others in that Office, and also by the writer for the New York Conservation Department, recently have substantiated and elaborated the following general scheme.

The aeciospores of *Cronartium ribicola* are dry, dusty, powdery, and not easily wet. The spores are produced in quantities like windblown pollen, with a moderate ability to hover in the air (5), and capable of long-distance dissemination by the wind. There seemed to be no reason why these spores could not be carried miles as easily as hundreds of feet. This long-distance dissemination was indicated by some evidence produced by McCubbin (6), Posey and York, Overholts, and the writer (12, 14), and was satisfactorily proved by Pennington (7, 9).

The urediniospores of this fungus, on the other hand, are moist, sticky, easily wettable, usually found in clumps when collected by aspirators, megaphone traps, glass slides, etc. They are not produced in quantity, are never seen in clouds visible in ordinary daylight, and are seen only as sparse points of light by the beam-of-light method. They do not hover long, but appear to sink relatively rapidly (5, 14). They are easily separated from each other, spattered about by rain, and disseminated by insects (11, 12, 14). They have the general characteristics, including the spiny coat, of insect-carried pollen. Studies made during the past few summers for the Conservation Department in New York have substantiated the observations of 1918, that urediniospores are disseminated for only relatively short distances under ordinary conditions. In fact, the writer in New York recently has been unable to find good evidence of distance dissemination of these spores, although in very moist seasons urediniospores from *Ribes nigrum* may be blown 2,000–3,000 feet. The urediniospores are often disseminated no farther than within the original bush or to neighboring bushes. Under normal weather conditions, in any infected *Ribes* bush, there can usually be found distinct generations of uredinia, including what is apparently the first generation resulting from the aeciospore infection (12).

The only change in the original conception of the function of the sporidia of *Cronartium ribicola* has been a tendency to shorten the distance they may be blown. They apparently can disseminate the rust for distances up to one and one-half miles, perhaps farther in extreme cases, but ordinarily not more than half a mile, and can cause damage of considerable amount only at little more than 500 ft. in most cases. The main factor determining the distance these sporidia are disseminated seems to be that of number alone, following the mathematical laws of chance. The more sporidia produced, the greater will be the chance that some will be carried to considerable distances. The danger of *Ribes nigrum* to nearby pine

stands lies in the abundance of sporulation, the myriads of sporidia produced by the fungus, on this host.

These studies have resulted in the following conception of the functions of the spore stages of *Cronartium ribicola*:

(1) The aecial stage not only initiates the cycle but, more important, is primarily the long-distance-dissemination stage.

(2) The uredinial stage is a local-intensification stage, a repeating stage, in which spores are formed that are incapable of long-distance dissemination but which produce an abundance of lesions locally, for the purpose of increasing the volume of telial material as a basis for the production of sporidia for infection of the pine.

(3) The telial stage is a combination of an emergency stage, which insures the continuance of the rust cycle by developing just prior to the death of the host tissue, and a multiplication stage, whereby the fungus apparently puts forth its entire available energy in producing a multi-seriate column of teliospores, each of which multiplies itself again in the formation of four sporidia.

(4) The sporidia are the agents of local, limited dissemination, but their chief function is the reinfection of pine to complete the cycle.

BLISTER-RUST YEARS IN THE ADIRONDACKS

It has been shown that most of the infections in the Adirondacks took place in 1911, 1913, 1916, and 1919 (13). Nearly half of the cankers were found to be upon 1919 wood; and in view of the fact that there were more cankers on 1918 and 1917 wood than wood of other years before or since 1919, it appeared that in all probability most of these cankers resulted from infections in 1919 in two- and three-year-old needles, even though artificial inoculations of three-year-old needles had proved unsuccessful (16). If such were the case, as seems likely, between 60 and 70 per cent of all cankers in the Adirondacks resulted from infections in 1919, a year known to have been unusually favorable for development of the blister-rust fungus. A study of the distribution of cankers by years shows the progress of the fungus as it becomes established, is disseminated, and increases in amount slowly and gradually, gathering momentum in the relatively unfavorable years, and literally showering the pines with an immense volume of sporidia in the favorable years.

The first really big blister-rust blast occurred in 1919. In previous years there had been nothing approaching it in severity, but it is beginning to appear now that the epidemic in some of the recent years may approach or even exceed it in places. This of course was to be expected.

If relative humidity is a factor in producing a severe blister-rust attack (15), it was expected that 1924 might be added to the list of years of heavy

blister-rust infections. There were, however, certain factors during 1924 operating against abundant infection of pine. The principal one was cold weather in the spring and early summer, which delayed the general infection of *Ribes* until late in July and the production of the telia in any considerable quantity until the last of August. Under ordinary conditions, in a moist season in the Adirondacks, the *Ribes* would be generally infected by the first of July; during July and August there would be a great increase in the uredinial stage of the rust, and an abundance of telia and sporidia after the first of Auguust. In 1924, however, the production of telia was decidedly limited. Observations in 1927 indicated that little infection took place in 1924.

It was predicted that 1925 would be another epidemic year. There was an unusually copious production of aeciospores in the spring, and the summer was rather moist and reasonably cool, especially during the period of telial production. During the latter part of the summer, teliospores were produced in abundance, and the stage appeared to be set for an epidemic.

Observations made in the early part of 1925 confirmed the general predictions. There are parts of the Adirondacks (especially the northern portion) where there was only a moderate amount of pine infection in 1925, but in other sections it appears that ultimately it may be proved that the epidemic of 1925 was more severe than that of 1919.

The summer of 1926 was rather dry and few infections of this year have been found. On the other hand, in 1927 there was an unnusual amount of precipitation in the Adirondacks, which provided several prolonged periods of high humidity favorable for infection of the pine (See 15). It is therefore expected that 1927 may also be looked back upon within the next few years as another one of the years of unusually heavy pine infection.

HISTORY OF BLISTER-RUST INFESTATIONS AS APPLIED TO CONDITIONS IN THE ADIRONDACKS

Inasmuch as 1919 was the year of greatest infection in the Adirondacks —at least as far as known definitely up to the present—there have been suggestions of the possibility that blister rust has reached the peak of its damage in that area. It may be possible that 1919 will prove to be the year of greatest pine infection in blister-rust history, although there is now room for considerable doubt as to this. But even so, the peak of the damage is quite another thing. It is difficult to conceive of such a thing so long as aeciospores are formed each spring, while *Ribes* exist near pine, and while seasons favorable for infection of pine occur periodically.

An attempt has been made to outline the history of blister rust in any given locality (13). The introduction of the disease by one or more wind-

blown aeciospores is called the first critical point. Following the introduction of the disease comes the intensification of the original aeciosporic infection by the uredinial stage (the local intensification stage) and, sooner or later, of course, infection of the local pines. Following local establishment on pine, a gradual increase in the amount of rust from year to year is inevitable until there is enough rust on pine to cause a general and heavy initial infection of *Ribes*, if not each year, at least in some very favorable year. This point is called the second critical point in the blister-rust history of a locality, and is attained as a result of dissemination and multiplication by the aecial stage and to a large extent by the intensive multiplication by the uredinial stage.

The third critical point is the initiation of the waves of infection which follow the general infection of the pine as above mentioned, when only favorable conditions for infection are required for great damage to pine. Each of these waves of infection does great damage to the pine; the younger the trees the more damage. The trees infected by the first great wave may or may not be damaged by subsequent waves, but infection at the earlier ages is always more likely to be fatal. Even though subsequent waves do no more damage to the trees as they become older, the young seedling reproduction continues to suffer with each succeeding wave. A peak in the damage can not be said to have been reached under these conditions.

The fourth critical point is the stage in which maturing trees begin to die from blister-rust attack.

The subsequent history of blister rust is a continuation of the destruction of trees of all sizes, even the oldest and over-mature, unless the matured trees are lumbered. As far as known, the momentum of this destruction continues as long as there are white pines to be killed, *Ribes* to carry the infective material, and conditions favorable for infection of the pine.

There is no evidence of acquired resistance or immunity of any sort in the pine or in any other gymnosperm. It is expected, as well as hoped, that some of the angiospermous trees such as the chestnut will develop a resistance to their diseases, but whether the more primitive gymnospermous organization could respond in such a way is more questionable.

One factor in the blister-rust complex, however, that may have considerable bearing upon the checking of the progress elaborated above is biological control. The rust fungus in the cankers on pine dies when the tree dies or the branch is shaded out. It may also die when it kills what appears to be hypersusceptible host tissue. The cankers on pine are sometimes destroyed in considerable numbers by rodents and other animals (14), and possibly by secondary fungi. The volume of aeciospores is certainly considerably reduced in certain seasons by these means, although it is probable that even

a severe reduction in the aecial stage would not affect the ultimate volume of sporidial production to an appreciable extent. The general functions of the uredinial and telial stages in the life cycle, as outlined above, seem adequate to provide for such an emergency. A few aeciospores, relatively speaking, can initiate a tremendous amount of blister rust in a season.

A more important phase of biological control concerns the *Ribes*. Inasmuch as *Ribes* are necessary for the spread of blister rust, anything which affects them must necessarily affect the amount of the disease on pine. The total potentially infectable leaf surface of *Ribes* may be seriously reduced by defoliation by the blister-rust fungus or by insects. Defoliation may be partial or complete, may be repeated once or twice a season, and may be repeated in two or three successive seasons. Successive defoliations during two or three seasons, or perhaps in one season, usually result in the death of the bushes (8). There are many stands of seriously-infected pine near which there are no *Ribes* because they undoubtedly had been killed in one way or another after the pines were infected. In the Kelm Mountain plot in 1924, it was found that 50 per cent of the existing footage of stem was devoid of leaves, and half of this, or 25 per cent of the total footage, was dead. In 1927, between 50 per cent and 75 per cent of the leaf-bearing stem of the *Ribes* was dead. At the Dannemora plantation in 1927, the cankers were for the most part from 1919 and 1921 infections with very few on 1925 wood. It was found that the bushes of *Ribes triste*, from which the infection came, bore only 6–15 leaves each on young and very short stems, and yet had very extensive and well established root systems. The original bushes had apparently been seriously damaged by blister rust, insects, or by other means.

Therefore it may be possible that the momentum of the white pine blister-rust depredations may be checked considerably by destruction of the Ribes; at least their elimination may mean periodic variations in severity of the rust and perhaps a permanent check under certain conditions. Under such conditions, if no more *Ribes* become established, a peak presumably might be reached in certain localities. Even at Kelm Mountain, however, new infections are taking place each favorable season.

The peak of the damage cannot be said to have been attained, however, when only 11 per cent of the trees studied are now dead and 19 per cent of the doomed trees are still alive (13). The damage from infections on the trees at the present time has not matured yet, and there will surely be more infection of the pine. At present, no striking amount of damage is visible in the Adirondacks: there are no large numbers of dead trees, no large trees dead. The Kelm Mountain lot, in which 90 per cent of the trees are infected and at least 87 per cent doomed, still looks like a fine, healthy stand of 18-year-old pine.

In the Adirondacks, the second and third critical points have been reached. Because of the scarcity of *Ribes* or absence of favorable infection conditions in certain sections, the pine will not be generally destroyed, and may not be damaged at all. But where there are *Ribes* present under normal conditions, the maximum damage has not been done. The Kelm Mountain area now shows what may confidently be predicted where *Ribes* occur, and the Horicon lot shows what may surely be expected. The possible damage is not yet complete at either place, although it is imminent, inevitable, and prognosticable.

The data available lead us to believe that, with the momentum gathered since 1919, the next severe blister-rust attacks will be much more formidable. As this is being written, the reports seem to indicate that the epidemics of 1925 and 1927 were of sufficient magnitude to bear out this statement. The concept of blister-rust momentum, the progressive and continuous gathering of increasing force through the years—which in this particular case means ultimately the volume of sporidia—has never been completely, thoroughly, and adequately grasped.

SQUIRREL INJURY TO CANKERS

After particularly severe winters, it has been reported by several observers (14) that many cankers are almost completely gnawed away, presumably by squirrels and perhaps by porcupines and other rodents. Notes were taken in the first place merely to satisfy a curiosity as to how large a percentage of the cankers was destroyed by animals. It is certain that in some seasons they consume a great deal of aecial tissue. They seldom entirely clean off the diseased area, but usually consume all the living affected bark except a narrow zone of about enough tissue to continue growth the next spring. These animals must cause considerable diminution in potential production of aeciospores, for they consume nearly all the portion of the diseased bark which would produce aecia the next spring. Of 11,100 cankers examined, 4,600 or 41 per cent had been gnawed by rodents, and in most cases the injury was complete enough to prevent the formation of aecia the following spring.

The animals eat the bark of cankers of all sizes of branches and trunks, from very small ones up to those as large as 13 in. in diameter (Fig. 1) according to the writer's observations. In many cases, it is noted that rodents have eaten the new growth of the same cankers each year. In 1925 a new type of injury was noted, namely, the cutting off of small cankered twigs entirely. The stub ends of the twigs bear rust-infected tissue. The explanation seems to be that the small twigs will not support the weight of the animal while it eats the bark, and therefore the twig is snipped off and eaten where it is more convenient.

FIG. 1. Blister-rust canker on white pine gnawed by rodents. Horicon Plot, Horicon, N. Y.

The canker is now 30 in. by 18 in. The tree is 15 in. D. B. H. and at the canker is 15¾ in. in diameter as seen in the photograph and only 11½ inches at right angles to the canker because of the flattening effect. The fungus entered the trunk on the adventitious shoot seen in the center of the canker. The decorticated portion of the canker was gnawed out by rodents in the winter of 1922–23 and the line of demarcation represents all but the narrow rim of the canker at that time. The ruler resting upon the adventitious shoot is 6 inches long.

This rodent injury was very common during the winters of 1922–1923 and 1923–1924 in the Adirondacks, but was almost entirely lacking during the three succeeding winters. Less of it has been noted in Essex and Clinton Counties than in Warren County.

INVASION OF BLISTER-RUST CANKERS BY SECONDARY FUNGI

The possible effect of secondary fungi in destroying blister-rust cankers or preventing fruiting has attracted the attention of several workers (1, p. 650; 14, p. 29). The data at hand show that 20 per cent of the cankers are invaded by secondary fungi. In the McPhillips No. 1 plot, 60 per cent of the cankers were infected with other fungi. In most cases the invasion is quite complete, and sporulation of the rust fungus is prevented if the canker is not already dead.

The precise biological significance of secondary fungi in cankers is unknown. Undoubtedly, many cankers are invaded where the aecia rupture the bark and expose the inner-bark cells. Colley has suggested the joint action of the rust fungus and the secondary fungus in completing the

girdling of the stem (1, p. 650). It has also been assumed by many of those interested that the significance of the secondary fungus rests in its prevention of fruiting by killing the living bark. It is true that in many cases cankers which have never borne aecia are found attacked by secondary fungi. These may have entered the canker before the bark was dead and aided in the killing, or they may have entered after the tissue was dead. The exact relations can be determined in very few cases, but it appears that either of these courses is possible.

The fungi most commonly found on cankers are a species of *Phoma* (imperfect stage of *Caliciopsis pinea*) and *Cenangium abietis* (Pers.) Rehm. An unidentified species of *Stereum* (perhaps *S. sanguinolentum* Alb. & Schw.) has also been found occasionally. In 1927 a new fungus was found to be very common in the Dannemora Plantation, both as a secondary fungus on blister rust cankers and to a less extent on bark not infected by *Cronartium ribicola*. It was identified[1] for the writer as *Dasyscypha agassizii* (B. & C.) Sacc. This is apparently the first time this fungus has been reported upon *Pinus strobus*.

MORTALITY OF BLISTER-RUST-INFECTED WHITE PINE IN RELATION TO AGE OF TREES AT TIME OF INFECTION

Data based upon the damage studies mentioned earlier (13) show that the older the pines are when they are infected, the less chance there is of their being killed. The discussion is resumed here for amplification and in order to present more data. The facts have excited a little criticism and skepticism, and while they probably are subject to variation under certain conditions, on the whole there is some meaning in them. The new data are incorporated with the old in figure 2.

Logic and certain facts support the thesis. The blister-rust fungus enters the tree for the most part through the needles of the branches, except in the earliest years. Most of the cankers on trees are originally branch cankers, and after the tree is five years of age this is true in over 98 per cent of the cases. The reason is obvious. The scattered, unprotected tops are exposed to drying, while the lower branches—bushier, more abundant, closer together, nearer the ground, and surrounded by other vegetation—are bathed by air of relative humidity high enough for infection, for much longer periods than the tops. Counts of over 15,000 cankers on from 12- to 20-year-old trees show that 98 per cent of them are less than 3 ft. from the ground, 95 per cent less than 2 ft. In other words, they are down in the thick lower foliage where the relative humidity sinks later in the day

[1] The fungus was first sent to Dr. E. A. Burt through an error, and later to Dr. F. J. Seaver. Both identified it as noted.

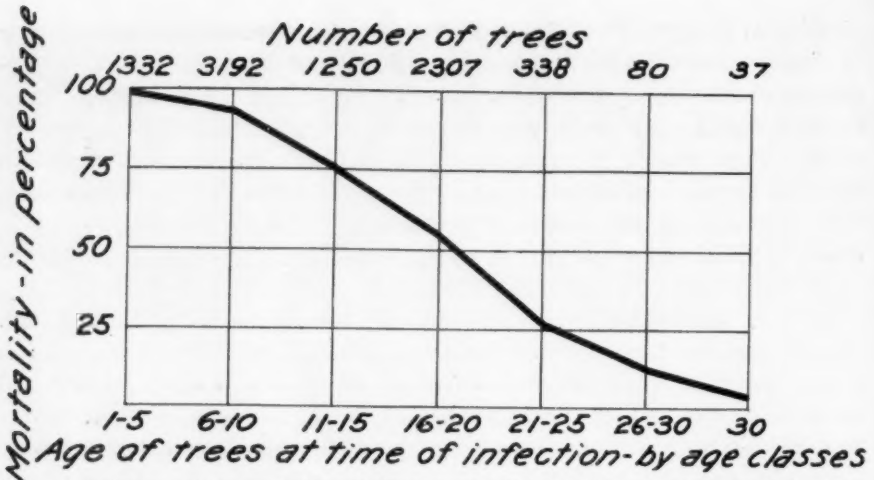
Number of trees
1332 3192 1250 2307 338 80 37
100
75
50
25
Mortality - in percentage
1-5 6-10 11-15 16-20 21-25 26-30 30
Age of trees at time of infection - by age classes

FIG. 2. Relation of the mortality of blister-rust-infected white pines to age of the trees at time of infection. Based on definite data from about 8,500 trees.

and rises earlier than up in the tops. Of the 2,134 cankers in the Dannemora Plantation and the 9,000 in the Kelm Mountain lot, only 1.1 per cent were in the open, unprotected by foliage.

Inasmuch, therefore, as the larger proportion of the cankers are branch cankers, and inasmuch as the needles on the branches persist only 3–5 years, it stands to reason that the difficulty encountered by the fungus in reaching the stem increases with the distance of the needles from the stem—that is, with the age of the tree, discounting the small number of top infections. In other words, the older the tree at the time it is infected, the greater the period of time consumed by the fungus in travelling from the place of infection on the branch to the stem where alone it can kill the tree; and the longer the fungus requires to reach the stem, the greater the opportunity for the tree to escape harm through the death of the fungus in the canker by shade, fungi, and insects or other animals. Point may be given to the foregoing statements by the following isolated facts: the recovery of 69 infected trees at Kelm Mountain since 1923 (meaning the death of cankers by shading so that the trees are no longer doomed to die); the dropping of the infection percentage at Kelm Mountain from 97 in 1923 to 90 in 1927, and the death of over half the 9,000 cankers in the lot by shading; the dead condition of 66 per cent of the cankers on the Whalen lot worked in 1925; and the 45 per cent of dead cankers on all the lots in the first studies with no accounting of conditions since.

The new data collected each year have substantiated the original results, with only slight changes in the mortality percentages for the different age classes.

RELATION OF PERCENTAGE OF INFECTION TO STOCKING OF STAND

Posey and Ford (10), in their paper on the infestation at Kittery Point, Maine, presented a curve showing that the degree of infection varies inversely as the stocking, apparently because of screening or some such cause, and in spite of the occurrence of moisture conditions more favorable for infection in the thicker stands. This general fact seems to hold true under certain conditions, especially where the infection is not heavy, but it is true in very few cases where there is sufficient infection of pine to provide a fair test.

When the infection percentages of the New York one- and two-acre plots were plotted in this way, the curve was a duplicate of the one given by Posey and Ford. But, on the other hand, in many of the plots with the heavily and the lightly stocked portions sharply separated, it was found that this relationship did not hold true except in a few cases. For example, at Kelm Mountain, with 97 per cent infection on the two acres, there was no difference in infection percentage whether the stocking was 3,000 to the acre or only 400. At Horicon, plots varying from ¼ to 1 acre in size with stockings of 600, 1,300, and 2,400 trees have 43 per cent infection, 41 per cent, and 80 per cent respectively. On the Whalen lot, a plot with 800 trees an acre had 63 per cent infection, while a plot with 2,500 trees an acre had 54 per cent. On the Sternberg lot, a plot with 4,800 trees an acre had 7 per cent infection and a plot with 8,960 an acre had 66 per cent infection.

All pertinent data obtainable in New York from the writer's work are represented in figure 3. It will be noted that, up to 3,500 trees an acre, the density of the stand apparently has no effect upon the degree of infection in the experimental plots. The plots with 4,000 or 5,000 trees an acre have a low percentage of infection, but a small plot, on the other hand, with 9,000 trees an acre (Sternberg B) has a high percentage. The Remington plots with 15,000 trees an acre have a low percentage of infection, but it is to be noted that the *Ribes* on this area are few in number and small in size. Furthermore, the pines have been exposed to infection in none of the important blister-rust years except 1925, and the damage resulting from infection in that year is not yet evident, if any great amount of damage could occur with so few *Ribes* as the source of infection.

One major criticism of attaching too much importance to this curve as representing a blister-rust generalization is that the data in any particular case are not complete. For example, in one of the Remington (Chestertown) centers in the graph, with 14,000 trees an acre, the infection was 6.4 per cent in 1925 and 15 per cent in 1927. With a large amount of *Ribes* instead of one or two scraggly bushes, this percentage would undoubtedly be higher even now. Too little is known of what happened to the

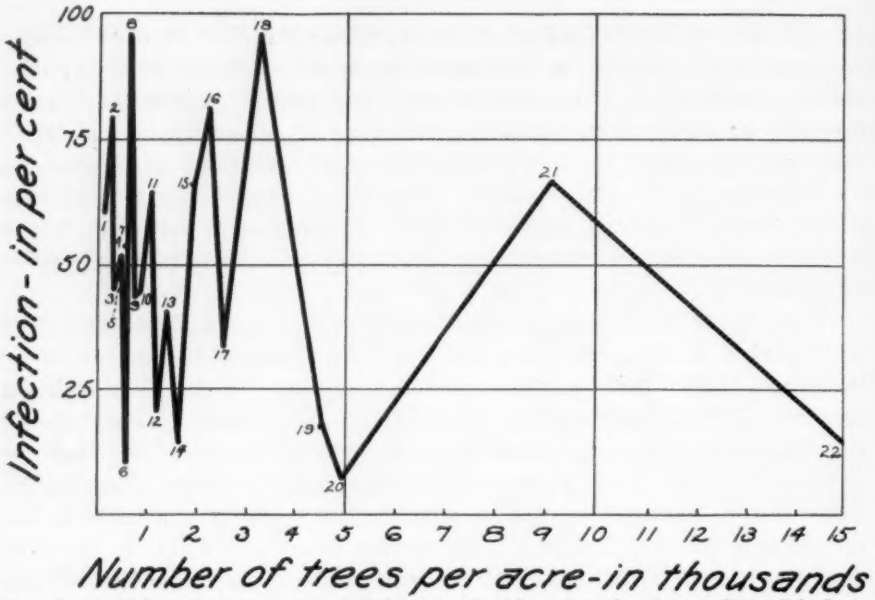

FIG. 3. Relation of percentage of infection of white pines by *Cronartium ribicola* to stocking of stand.

The numbered points on the graph represent the percentages pertaining to the following plots: 1—Myers; 2—Harkness; 3—American Steele and Wire Co.; 4—Goff; 5—Brown (the point ought to be nearer the 50 line than can be shown here); 6—McPhillips No. 2; 7—Downes; 8—Kelm Mt.; 9—Horicon No. 1; 10—Ames; 11—Whalen; 12—Smith; 13—Horicon No. 2; 14—Burdick; 15—McPhillips No. 1 (1924); 16—Horicon A; 17—McPhillips No. 1 (1926); 18—Kelm Mt. A; 19—Sternberg A; 20—Sternberg; 21—Sternberg B; 22—Remington.

reproduction on many of our New York plots just following the great 1919 blast. It is known that the Horicon plot had a large quantity of young reproduction in 1920, which was not there when the plot was studied in 1923. Our observations of many other lots, including Kelm Mountain plot, lead us to believe that similar conditions existed on those. The two-storied condition[2] of most of the New York plots show that our figures of stocking of stands represent only present conditions. The writer can never be convinced that any high degree of stocking would have kept the percentage of infection very low on the Kelm Mountain, Horicon, Whalen, Downes, Harkness, and McPhillips lots (13). It seems reasonable to assume that

[2] By "two-storied condition" is meant the presence upon the lots of trees under 10 years of age and over 17 years of age, without any between these limits. The younger class of trees came in after 1919 and the older class prior to 1911, while the series of great waves of infection from 1911 to 1919 inclusive destroyed most of the seedlings which started during this period. (See 13.)

a large percentage of the countless seedlings lost long before any studies were made were destroyed by blister-rust, and that if knowledge of these conditions were at hand the percentages of infection for these lots would be much higher than is shown by the record.

DEAD CANKERS

As a result of counts made of the number of dead cankers, it was found that 45 per cent of all cankers on the plots, on both dead and living trees, are now dead. They are dead either from injury by rodents, invasion by secondary fungi, killing of the bark by the blister-rust fungus itself, or shading out. On the Whalen lot, 57 per cent of the 1,500 cankers were dead at the time of study in 1925.

INSECTS, RODENTS, SLUGS, AND SNAILS FEEDING UPON PYCNIAL DROPS

The pycnial drops and pycnial tissues of blister rust cankers are sweet. The sugar present at the time of examination was found by Dr. Nathaniel O. Howard of the Office of Forest Pathology, U. S. Department of Agriculture, to be glucose. It has been assumed that squirrels and other animals eat the resinous pine bark partly because of its succulence, when other food is unavailable on account of snow. On the other hand, porcupines will eat extensive areas of healthy pine bark in some winters, without apparent regard for the resin. A recent paper by Hosley (4) reports that, in certain localities when the snow is deep, red squirrels have been found eating the buds of several coniferous trees, including white pine, and that they clip off the terminal shoots of white pines. Although it is not made clear in the paper, the squirrels apparently remove the terminal shoots of the white pine for the buds and not for the bark. It appears to be the succulence of the bud tissues that appeals to the squirrels, in spite of the resin, although it is not known whether the buds are sweeter than the normal bark tissue.

But whatever may be the attractive element in the pycnial zone of the bark, rodents have developed a liking for the sweet pycnial fluid. No squirrels have been observed feeding upon the pycnial drops themselves, but paired, chisel-like teeth-marks show that they scrape off the pycnia before the fluid has been exuded. In 1926 and 1927 it was found that the entire crop of pycnia in certain localities had been consumed.

It was not until 1924, however, that the writer noticed that the pycnial drops attracted insects in any considerable number. In 1924 and 1925 the pycnial drops were large and abundant because of the moistness of the seasons. Various hymenopterous insects such as ants, bees, and hornets were commonly observed feeding on the drops as long as they persisted, and also certain Hemiptera, Coleoptera, and Lepidoptera. The insects industriously and thoroughly cleaned up the pycnial areas, drop by drop.

Slugs and small snails also have been observed feeding upon the pycnial drops, especially in moist seasons such as 1924 and 1925.

SLUGS AND SNAILS FEEDING UPON AECIOSPORES

Different kinds of insects have been found upon the aecia of *Cronartium ribicola,* apparently in search of food. Insects and other animals have been found carrying aeciospores on their bodies (2, 3, 10, 14). It was not until 1927, however, that the writer observed animals eating aeciospores. In 1927 at Dannemora, early in the morning, or all day during wet weather, slugs and small snails were found feeding upon the aeciospores all through the season of aeciospore production, which lasted until July 17. They were seen with their mouth parts in the aecia like pigs at a trough, and when they were removed to a little distance they invariably returned to that position forthwith. Furthermore, aeciospores were found in the alimentary tracts of slugs captured on the aecia, and the feces of these slugs showed remains of aeciospore walls (See 3).

SUMMARY

1. This paper presents observations made in connection with studies on the damage caused by white pine blister rust, carried on in the Adirondacks for the New York Conservation Department.

2. The functions of the different spore stages of the blister-rust fungus are discussed from a biological point of view.

3. In the Adirondacks, between 60 per cent and 70 per cent of the cankers initiated prior to 1925 were on 1919 wood. The epidemic of 1925 appears to have been next in severity to that of 1919, if not more severe. The season of 1927 likewise appeared to be favorable for heavy pine infection.

4. In the life history of blister-rust there are four critical points. During the later phases, biological control may operate. In the Adirondacks, however, on account of the epidemics of 1925 and 1927, blister rust undoubtedly has not reached its peak.

5. During years unfavorable for blister rust, momentum is acquired for the voluminous showering of sporidia on the pines when conditions are favorable. The importance of this momentum has not heretofore been appreciated.

6. A certain amount of inoculum is destroyed by small animals that eat the cankers and by attacks of secondary fungi. *Dasyscypha agassizii* (B. and C.) Sacc. is reported on blister-rust cankers and on *Pinus strobus* for the first time.

7. Mortality of infected pine trees decreases with the age of the trees at the time of infection.

8. While the percentage of infection appears, in general, to vary inversely as the stocking of the stand, this tendency is apparent only because of unfavorable infection conditions in a small lot of young trees with 15,000 to an acre.

9. Forty-five per cent of the cankers on the experimental plots are now dead. On one lot, 57 per cent were dead in 1925.

10. Rodents, slugs, snails, and many insects feed on pycnia and pycnial drops.

11. Slugs and snails feed on the aeciospores.

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BORDEAUX SPRAY IN THE CONTROL OF FIRE BLIGHT OF APPLE[1]

MONROE MCCOWN[2]

Owing to the destructiveness in Indiana orchards of fire blight, caused by *Bacillus amylovorus,* and the fact that beneficial results from the use of a bordeaux spray have been observed by investigators and midwest orchard men, spraying tests and laboratory studies have been carried out at Lafayette and in Indiana orchards. The results of the field tests show that a comparatively weak bordeaux spray in the full-bloom period reduced the amount of blossom infection in Grimes and Jonathan apples. In the laboratory, bordeaux mixture and copper dusts apparently killed the bacteria. It is the purpose of this paper to present more in detail the evidence upon which was based a report[3] recently made to the Indiana fruit growers.

THE EFFECT OF A FULL-BLOOM BORDEAUX SPRAY UPON BLOSSOM INFECTION

On April 28, 1927, 100 clusters of blossoms on a Grimes tree in full bloom in the University orchard at Lafayette were sprayed once by means of an atomizer with bordeaux 1–3–50 (1 lb. $CuSO_4$, 3 lbs. hydrated lime, 50 gals. of water). Immediately after the bordeaux application, 50 of these clusters were inoculated by means of an atomizer containing a cloudy suspension of the fire-blight bacteria in distilled water. Of the remaining 50 clusters, 25 were similarly inoculated 24 hours later (April 29), and the remaining 25 inoculated 48 hours after the bordeaux was applied (April 30). Each day 25 unsprayed blossom clusters were inoculated as checks.

The inoculations were made between 1:30 and 3:00 P. M. The maximum temperatures as reported by the U. S. Weather Bureau for April 28, 29, and 30 were 69°, 68°, and 61° F., respectively. Following

[1] Contribution from the Department of Horticulture, Purdue University Agricultural Experiment Station, Lafayette, Indiana. Part of the data in this paper was presented to the Faculty of Purdue University, May, 1928, as a thesis in partial fulfillment of the requirements for the degree of Master of Science in Agriculture.

[2] The writer is indebted to Dr. Max W. Gardner, of the Department of Botany, Purdue University Agricultural Experiment Station, for laboratory methods and valuable advice and suggestions during the progress of the work; to Professor F. P. Cullinan, of the Department of Horticulture, and Professor P. H. Brewer, of the Department of Agronomy, for suggestions and advice; and to the Department of Botany, Agricultural Experiment Station, for laboratory facilities.

[3] MCCOWN, MONROE. Spraying for the control of fire blight in the apple. Trans. Ind. Hort. Soc. 67: 129–133. 1928.

the spray application, and prior to the inoculations of April 29, 0.36 in. of rainfall was recorded. Those clusters inoculated April 30 were subjected to an additional 0.32 in. of rainfall prior to inoculation. On April 28, when the spray was applied, there was an average of one unopened blossom in each cluster. The significance of this unprotected blossom will be shown later.

The clusters were watched closely and all infected blossoms were removed at intervals of two to three days and examined under the microscope to verify the diagnosis. The bacteria ooze out in conspicuous masses from the cut pedicels of blighted blossoms which are sectioned and mounted in water under the microscope. Two types of blossom infection were recognized: primary, in which the bacteria had entered presumably through the nectary; and secondary, in which the bacteria had entered the pedicel from the spur.

TABLE 1.—*Effect of bordeaux mixture 1–3–50 applied as a full-bloom spray upon the number of fire-blight infections in Grimes apple blossoms*

Time of inoculation, 1927,	Spray treatment	No. blossom clusters	Av. no. primarily-infected blossoms a cluster	Percentage of clusters infected
April 28, immediately after spraying	Bordeaux 1–3–50[a]	48	0.08	8
	None (Check)	24	1.46	87
April 29, 24 hours after spraying	Bordeaux 1–3–50	24	1.08	71
	None (Check)	24	2.24	96
April 30, 48 hours after spraying	Bordeaux 1–3–50	24	1.25	75
	None (Check)	25	1.88	88

[a] Bordeaux mixture made up at the rate of 1 lb. $CuSO_4$ and 3 lbs. hydrated lime to 50 gals. water.

The results, recorded in table 1, show that bordeaux 1–3–50 was very effective against blossom infection when the clusters were inoculated immediately following the bordeaux application. In this case, only 8 per cent of the sprayed clusters developed infection, while 87 per cent of the unsprayed check clusters became infected. Those clusters, inoculated 24 hours following the spray application, and after a rainfall of 0.36 in.,

developed considerably fewer infections than the checks. Only 71 per cent of the sprayed clusters developed infection, while 96 per cent of the check clusters blighted. While 75 per cent of those clusters inoculated 48 hours after spraying blighted, 88 per cent of the check clusters inoculated at the same time were infected. These clusters had been subjected to an additional rainfall of 0.32 in. prior to inoculation. No natural infections occurred in this tree.

A comparison of the number of primarily infected flowers in the sprayed and unsprayed clusters shows an even more striking difference than do the relative percentages of clusters infected. However, from a practical standpoint this means little, for, if one flower in a cluster develops primary infection, in all probability the remaining flowers in that cluster will be killed by secondary infection. Apparently the bacteria travel down the pedicel of the primarily infected blossom into the spur and up through the stems of the remaining flowers.

Upon first thought one might be inclined to attribute the poorer control obtained in those clusters inoculated 24 and 48 hours after spraying entirely to washing away and dilution of the bordeaux in the blossoms by the intervening rains. Because of the marked reduction in primary infection in these clusters, it would seem, however, that the one unprotected blossom in each cluster might account for much of this increased infection. Previous mention was made of the fact that at the time of spraying there was an average of one unopened blossom in each cluster. These blossoms opened between the time of spraying and the time of the two later inoculations and contained none of the protective spray material.

Spraying tests[4] were carried out in a number of Indiana orchards in 1928, a season in which fire blight was particularly prevalent.

On May 2, 1928, ten mature Jonathan trees, in the Experiment Station orchard located near Bedford, were sprayed once, when in full blossom, with bordeaux 1–3–50. Counts made on June 16 of the number of infected blossom clusters on each of the ten sprayed trees and seven unsprayed check trees showed a reduction of 79 per cent due to spraying. There was an average of only 68 blighted blossom clusters a tree on the sprayed trees, while the unsprayed-check trees averaged 329 infected clusters a tree. The number of infected clusters on the sprayed trees ranged from 4 to 187, and on the unsprayed checks, 171 to 938.

[4] The writer wishes to acknowledge the cooperation of Dr. B. A. Porter, U. S. Bureau of Entomology, Vincennes, Indiana, who applied the spray in the Vincennes test in connection with some of his own investigations; and of Mr. Hobert Hall, Superintendent of the Moses Fell Annex Farm, Bedford, Indiana, who made the full-bloom application in that orchard.

A similar test was made on Jonathan trees in an orchard near Vincennes. A count made of the number of blighted clusters a tree on June 5, on four representative sprayed trees and three check trees, showed a reduction of 52 per cent due to the one bordeaux application. The four sprayed trees averaged 209 infected blossom clusters a tree, while the three check trees contained an average of 440 blighted clusters each. The spray, bordeaux 1–3–50, was applied May 1, when the trees were just coming into full blossom.

In a small test, involving three sprayed pear trees and one unsprayed tree in an orchard near Lafayette, the number of infected blossom clusters a tree was reduced 98 per cent. The three sprayed trees contained an average of only 10 infected clusters a tree, while there were 474 blighted clusters in the check tree when the counts were made on June 2. The one spray application of bordeaux 1–3–50 was made May 3, when approximately 80 per cent of the blossoms were open. In all of these tests, the only departure from the recommended spray schedule was the addition of the one full-bloom spray. No bordeaux was used in any of the other early applications.

These results, showing that bordeaux reduces blossom infection, are in accord with the findings of McCue[5] in Delaware, who, in 1907, sprayed five Bartlett pear trees with bordeaux when in full blossom with a consequent reduction in the number of infections. Stevens, Ruth, Peltier, and Malloch,[6] in Illinois, reported complete control of fire blight infection in two Kieffer pear trees by spraying with bordeaux through the period of blossoming and for some time thereafter. Although the conditions are not exactly comparable, it is of interest to note that Reimer,[7] who worked with five varieties of pears in Oregon, concluded after three years' investigation that bordeaux would materially reduce the number of blight infections.

No russetting of the fruit occurred in any of the Indiana tests mentioned above. The same strength of bordeaux has been used as a full-bloom spray in other orchards in Indiana during the past three years and in no case has serious russetting resulted. Furthermore, no reduction in set of fruit has been observed in any of the tests. No arsenic was used in any of the tests.

LABORATORY TESTS

Weak Alkaline Bordeaux

Laboratory studies were begun in 1926 to determine the effect of bordeaux in various forms upon the fire-blight bacteria. Bordeaux was made

[5] MCCUE, C. A. Pear blight. Trans. Peninsula Hort. Soc. **1917**: 51–55. 1917.

[6] STEVENS, F. L., W. A. RUTH, G. L. PELTIER, and J. R. MALLOCH. Observations on pear blight in Illinois. Trans. Ill. State Hort. Soc. **50** (1916): 216–227. 1917.

[7] REIMER, F. C. Value of bordeaux mixture in blight control. Trans. Ore. State Hort. Soc. **17** (1925): 136–142. 1926.

up from a stock solution of $CuSO_4$ and a stock suspension of hydrated lime. In some cases these stock preparations were sterilized by heating in an autoclave, but this procedure was found to be unnecessary. In each of a series of test tubes, 3 cc. of bordeaux were made up at such a strength that the addition, to each tube, of 1 cc. of a cloudy suspension of the fire-blight bacteria (from pure culture of proved pathogenicity) in sterilized distilled water just brought the bordeaux up to the strength desired. At specified time intervals, following the introduction of the bacteria, loop inoculations were made from the bordeaux tubes into tubes of melted potato agar or beef-dextrose agar and plates were poured. For a control, one or more plates were poured from tubes of agar inoculated with a loopful of the bacterial suspension diluted one to three with sterilized distilled water. The colonies that developed in these plates were counted.

The results of three series of laboratory tests with bordeaux, of strengths varying from 1/4–3/4–50 to 1/64–3/64–50, are presented in table 2. These

TABLE 2.—*Effect of liquid bordeaux upon the fire-blight bacteria when the bacteria are suspended in the mixture and exposed to its action for varying periods*

Series	Suspension medium	No. colonies produced on poured agar plates after exposures indicated				
		2 min.	4 min.	6 min.	12 min.	24 min.
	Water (Check)	.....	....	....	....	12,000
A	Bordeaux 1/4–3/4–50[a]	960	....	0	0	0
	do 1/8–3/8–50	9,500	....	44	0	0
	do 1/16–3/16–50	10,000	....	194	0	0
	Water (Check)	.....	....	....	....	15,000
B	Bordeaux 1/16–3/16–50	13,000	....	0	0	0
	do 1/32–3/32–50	12,000	....	5	0	0
	do 1/64–3/64–50	14,000	....	1	0	0
	Water (Check)	.....	....	....	....	4,500
C	Bordeaux 1/8–3/8–50	162	43	0	0	0
	do 1/16–3/16–50	37	2	0	2	0

[a] Bordeaux mixture made up at the rate of ¼ lb. $CuSO_4$ and ¾ lb. hydrated lime to 50 gals. water.

results show that bordeaux in the liquid state apparently killed the bacteria after comparatively short exposures. Very few colonies were produced in those plates poured after an exposure of 6 minutes and in no case were colonies found in those poured after an exposure of 24 minutes. It is also

interesting to note that the weaker mixtures of bordeaux were apparently as effective as the stronger ones.

Dilute Lime Water

It was known that the bordeaux used in the above tests was highly alkaline. The effect of lime water upon the bacteria was next tested in an effort to determine whether the apparent killing might be due to the action of the hydroxyl ion.

To make up lime water that would be fairly comparable to the concentration of lime in a 1/4–3/4–50 bordeaux, hydrated lime was added to water at the rate of ½ lb. to 37½ gals. of water. One cc. of a cloudy suspension of the fire blight bacteria in sterilized water was then added to three cc. of the lime water. The resulting strength of the lime water was equivalent to ½ lb. of hydrated lime to 50 gals. of water. Plates were poured as in the tests with alkaline bordeaux previously described, and the colonies that developed in the plates were counted. The results of two series of tests with lime water are recorded in table 3.

TABLE 3.—*Effect of lime water upon the fire-blight bacteria when the bacteria are suspended in the solution and exposed to its action for varying periods*

Series	Suspension medium	No. colonies produced on poured agar plates after exposures indicated						
		2 min.	6 min.	12 min.	24 min.	30 min.	45 min.	50 min.
	Water (average of 3 plates)	.....	.....	.....	.....	.....	.....	15,000
A	Lime water (½ lb. to 50 gals. water	16,000	2,000	1,200	11,000	207	298	142
B	do	5,330	3,300	3,150	145	289	7	0

While the lime water apparently killed or inactivated the bacteria, the action was much slower than that of the liquid bordeaux. This slower action of the lime water suggested the presence of some toxic factor other than the hydroxyl ion in the liquid bordeaux.

Neutral Bordeaux

Neutral bordeaux was made up by adding lime water to a solution of copper sulphate until the resulting suspension showed a pH of approximately 7.2 when tested with brom thymol blue. The bordeaux was tested with potassium ferro-cyanide and no free copper found to be present. Only

enough lime was used to neutralize the copper sulphate and insure the absence of any free copper. The method of testing the effect of this bordeaux upon the bacteria was the same as that used for the alkaline bordeaux. One cc. of a suspension of the bacteria in sterilized water was added to three cc. of the bordeaux, which then contained the equivalent of ¼ lb. of $CuSO_4$ to 50 gals. of water, and plates were poured at definite time intervals. Five series of plates were poured. In no case were colonies produced after an exposure of 45 minutes, and in most cases no living bacteria were found after an exposure of 24 minutes to the bordeaux. The checks showed normal growth. Neutral bordeaux, therefore, was more toxic than lime water, though not quite so toxic as the alkaline bordeaux. These results indicate that the apparent killing power of the liquid bordeaux is not all due to the action of the hydroxyl ion. The action of this ion alone, at a concentration of pH 7.2, could scarcely account for the toxicity shown in these tests.

Aluminium Hydroxide

If the copper ion is inactive in bordeaux as long as there is an excess of lime present, the question arises as to whether the colloidal films in the bordeaux might not inactivate the bacteria by adsorption. To test this hypothesis, aluminium was substituted for copper in the bordeaux formula.

Lime water was added to a solution of aluminium sulphate until the pH of the supernatant liquid was approximately 7.0. The suspension was made up in such a strength that after the addition of 1 cc. of a suspension of the fire-blight bacteria in sterilized water to 3 cc. of the aluminium hydroxide, it contained the equivalent of ¼ lb. of the aluminium sulphate to 50 gals. of water. This material was tested in the same manner as the bordeaux in the previous tests.

No reduction in the number of the bacterial colonies produced on poured-agar plates occurred in the three series of tests after exposures ranging from 2 to 60 minutes. This indicated that no killing or inactivation could be attributed to adsorption by the colloidal films. These results, together with the fact that neutral bordeaux was effective, indicate that the copper itself may possibly kill the bacteria even though the copper is present in a colloidal state.

Dried Bordeaux Films

Known strengths of bordeaux were made up and, by means of a pipette, 0.3 cc. was placed upon each of eight microscope slides in sterilized petri dishes. The drops were allowed to dry for 24 hours. The covers were partially lifted from some of the dishes in order to expose the drop to the air in the laboratory during the drying period. The other dishes were left covered and the drops on these slides dried much more slowly.

At the end of the 24-hour period, 0.3 cc. of a cloudy suspension of the fire-blight bacteria in distilled water was placed on the area occupied by the dried film on each slide. From each of these drops, one loopful was plated out at specified time intervals. Similar loopfuls of the original suspension were plated as checks.

The results of two series of tests, as recorded in table 4, show that all

TABLE 4.—*Effect of dried bordeaux films upon the fire-blight bacteria*

Spray material on slide	Method of drying	No. colonies produced on poured agar plates after exposures indicated				
		5 min.	10 min.	20 min.	40 min.	60 min.
None (check)	.....	.....	.....	.....	.....	30,000
Bordeaux 1/16–3/16–50[a]	Covered	3,500	740	0	0	0
do	Exposed	18,000	15,000	540	0	0
Bordeaux 1/4–3/4–50	Covered	20,000	1,600	0	0	0
do	Exposed	24,000	18,000	3,800	0	0
None (check)	.....	.....	.....	.....	.....	33,000
Bordeaux 1/16–3/16–50	Covered	17,000	900	0	0	0
do	Exposed	27,000	25,000	6,000	133	0
Bordeaux 1/4–3/4–50	Covered	21,000	500	0	0	0
do	Exposed	26,000	21,000	4,000	0	0

[a] Bordeaux mixture made up at the rate of 1/16 lb. copper sulphate and 3/16 lb. hydrated lime to 50 gals. water.

bacteria were killed after a one-hour exposure to the films and after a 20-minute exposure to all the films dried slowly in the covered dishes. It is interesting to note that the bordeaux which was allowed to dry inclosed in the covered petri dishes was more effective than that which dried exposed to the air in the room, and closely approximated in toxicity the alkaline bordeaux tested in the liquid state. When the drops of bacterial suspension were placed upon the dried films, it was noted that those films which had dried in the closed dishes became re-suspended in a form closely resembling the freshly prepared bordeaux, whereas, those which dried exposed to the air in the laboratory remained attached to the slides.

Copper Dusts

Certain fungicidal dusts were tested by dusting flamed microscope slides by means of small insect-powder guns. The slides, while held in a vertical position, were tapped gently to remove any excess of dust and then placed in petri-dish damp chambers. A large drop of a cloudy suspension of the bacteria in sterilized water was placed on each dusted slide. Plates

were poured at definite time intervals from tubes of agar inoculated with a loopful from the drops on the slides. Similar loopfuls from the original bacterial suspension were plated as checks.

The bacteria were killed after the following exposures to the various dusts: bordeaux dust, containing 11 per cent metallic copper, 40 minutes; colloidal copper-lime dust, containing 15 per cent metallic copper, 20 minutes; Holland's basic copper sulphate, diluted with an equal amount of hydrated lime, 40 minutes; Kiltone dust, containing 8 per cent metallic copper, 40 minutes; copper-lime dusts, containing 20 per cent, 25 per cent and 27 per cent dehydrated copper sulphate, 40–60 minutes. Possibly copper dusts would prove as effective as liquid bordeaux in the prevention of blossom infection.

SUMMARY

Inoculation tests showed that a weak bordeaux spray (1–3–50), applied in the full-bloom period, reduced fire-blight infection in Grimes clusters inoculated immediately after spraying. Only 8 per cent of the sprayed clusters were infected, while 87 per cent of the check clusters blighted.

Bordeaux 1–3–50 applied when the clusters were opening into full bloom reduced natural infection 79 per cent and 52 per cent, respectively, in two field tests with the Jonathan apple and 98 per cent in one test on pear.

In the laboratory, alkaline bordeaux in strengths varying from 1/4–3/4–50 to 1/64–3/64–50 killed the bacteria after short exposures.

A weak lime water ($\frac{1}{2}$ lb. to 50 gals. of water) killed the bacteria, though not so quickly as the bordeaux. This indicated that the toxicity of the alkaline bordeaux might be due, at least in part, to the action of the hydroxyl ion.

Neutral bordeaux (pH 7.2) was found to be almost as effective as the alkaline bordeaux against the organism in laboratory tests, a fact indicating that some toxic factor other than the hydroxyl ion was present.

The fact that alminium hydroxide did not kill the bacteria in similar tests indicated that the effectiveness of the bordeaux could not be attributed to adsorption of the bacteria by the colloidal films.

When drops of a bacterial suspension were placed on bordeaux films dried on slides, the organisms were promptly killed. It was found that those films that were dried while inclosed within petri dishes apparently became resuspended upon the addition of the bacterial suspension and were more effective than were the films dried while exposed to the air in the laboratory. Films of copper-lime dusts likewise killed the bacteria.

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VIABILITY OF CERTAIN PLANT PATHOGENES IN SOILS

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Comparatively little is known about the overwintering and longevity of plant-pathogenic bacteria in the soil. Much time and effort have been devoted to a study of the behavior of the organisms in the plant and in pure culture, but the biology of these organisms in the soil has been largely neglected. This is probably due to the difficulties encountered in isolating plant pathogenes from field soils. The medium devised and described by the author (11), utilizing crystal violet bile agar has, in a measure, overcome this obstacle in such studies of plant pathogenic bacteria.

In this paper it is proposed to describe the results obtained on overwintering of *Pseudomonas tumefaciens* Sm. and Town. and seven other plant pathogenic bacteria in sterilized and unsterilized soil kept in the laboratory and in the open.

The findings of other workers are briefly as follows. Rand and Enlows (14), while studying the longevity of *Bacillus tracheiphilus* Sm., showed that the organism did not overwinter in the soil. Anderson and Chapman (2), working with *Pseudomonas tabacum* Wolf and Foster, found that freezing of the pathogene on agar streaks did not affect its viability. They also showed that infested soil was one of the main sources of infection to a new crop. Their field experiments indicated that the wildfire organism was able to survive the winter in the soil. Later, Anderson (1) found that the agar cultures of the pathogene survived a very severe winter in New England. He also showed that the bacteria overwintered in sterilized soils in the open, but apparently not in unsterilized soil. Clinton and McCormick (5) also showed that the direct application of overwintered infested soils to tobacco plants produced typical infection in one out of several trials. While studying the same pathogene, Johnson and Murwin (7) found that it might live over winter in dry soil or in dry leaves, but not in moist soil nor in decaying leaves.

Fromme and Wingard (6), working with *Ps. angulatum* Fromme and Murray, found that the pathogene overwintered in the soil in the open in the state of Virginia.

[1] The writer wishes to express his sincere gratitude to Dr. I. E. Melhus and Dr. J. H. Muncie for their valuable suggestions and criticisms made during the entire period of this work. These studies have been carried out in connection with the joint crowngall project of the Crop Protection Institute of the National Research Council; United States Department of Agriculture, Office of Mycology and Disease Survey; Iowa State College; and University of Wisconsin.

Clayton (4) was unable to demonstrate that *Ps. campestris* (Pam.) Sm. lives over winter in the soil in New York state. Kendrick (8), while studying *Ps. holci* Kendrick, noted that the organism was virulent after 50 days' freezing in the refrigerator. His field observations suggested that the organism overwintered in the soil. Skoric (15) was unable to show the overwintering of *Ps. pisi* Sackett in field soils in the open.

McCulloch (9) obtained infections on healthy treated gladioli by planting the corms in soil where the disease caused by *Ps. marginatum* McC. had been prevalent for several years. Muncie (10) has shown that *Ps. phaseoli* Sm. overwintered in sterilized soil in Michigan.

Probably the writer was the first to isolate *Ps. tumefaciens* from a composite sample of infested field soil taken in a nursery. He (12, 13) also showed that the crown-gall pathogene overwintered in field soils, which fact has recently been confirmed by Banfield (3).

ISOLATIONS AND INOCULATION TRIALS WITH EIGHT BACTERIAL PLANT PATHOGENES

The author (13) has already shown that *Pseudomonas tumefaciens* may live 16 months in unsterilized soils. Later, further experiments were carried on to determine by means of isolations and inoculations the longevity of *Ps. tumefaciens,* when carried in unsterilized and sterilized soils of various types in the laboratory.

Two sets of soil samples placed in flasks, including sterilized and unsterilized clay, loam, and sand were infested on June 3, 1926, with a 72-hour broth culture of the crown-gall pathogene. One set of flasks was sealed with paraffin and kept at room temperature, while the other, unsealed, was kept in the refrigerator. The isolations were made from the unsealed set at frequent intervals, while the sealed set was opened for the first time after 480 days from the time of infestation. Since the soil was fairly dry in the sealed set, sterile distilled water was added. The method of isolation was the same as previously employed by the writer (11). The results of isolations and inoculations from both sets of soils made at irregular intervals are given in table 1.

It will be seen from table 1 that virulent crown-gall bacteria were recovered from sealed and unsealed samples of infested sterilized clay, loam, and sand 736 days after infestation. Tomato plants were infected when inoculated with organisms which were isolated from the unsterilized soils in sealed flasks. Isolations were made from clay after 539 days, and from loam and sand after 669 days. In five trials organisms from unsealed, unsterilized clay produced no infection, while those from loam and sand produced infection after 587 days. These data indicate that *Ps. tumefaciens* may live in a virulent state for a long time in sterilized and unsterilized

TABLE 1.—*Longevity of Pseudomonas tumefaciens in sterilized and unsterilized soils, infested on June 3, 1928, and kept in the laboratory, as determined by inoculations of tomato plants with suspected plate colonies*

Date of isolations	No. days organisms in the soil	Tomato plants infected[a] with suspected colonies from infested soils												
		Sealed						Unsealed[b]						
		Sterilized			Unsterilized			Sterilized			Unsterilized			Check
		Clay	Loam	Sand	Clay	Loam	Sand	Clay	Loam	Sand	Clay	Loam	Sand	
Sept. 24, 1927	480	+	+	+	−−	−−	−−	+	+	+	−	−	−	−
Nov. 21, 1927	539	+	+	+	+	−	+	+	+	+	−	−	−	−
Jan. 8, 1928	587	+	+	+	−	−	+	+	+	+	−	+	+	−
Feb. 27, 1928	637	−−	−−	+	−	−	−	+	−−	−−	−	−	−	−
Mar. 30, 1928	669	+	+	−−	−	+	+	+	+	+	−	−	−	−
Apr. 10, 1928	680	+	+	+	−	−	−	+	+	+	−	−	−	−
Apr. 27, 1928	697	+	+	+	−	−	−	+	+	+	−	−	−	−
May 3, 1928	703	+	+	+	−	−	−	+	+	+	−	−	−	−
May 5, 1928	705	+	+	+	−	−	−	+	+	−	−	−	−	−
June 5, 1928	736	+	+	+	−	−	−	+	+	+	−	−	−	−

[a] + = Galls produced.
−− = No isolations made.
− = No infections obtained.

[b] Previous studies were reported by the writer (13, p. 335, table 1).

soils. The organism lives longer in unsterilized sand than in clay or loam.

The fact that the crown-gall pathogene overwinters in the soil suggested the advisability of determining whether other plant pathogenes could overwinter in the soil. In these studies, cultures of eight bacterial plant pathogenes were employed, namely, *Ps. tumefaciens, Ps. marginatum, Ps. phaseoli, Ps. beticolum* Sm., Brown and Town., *Bacillus carotovorus* Jones, *B. atrosepticus* van Hall, *Ps. tabacum,* and *Ps. citriputealis* C. O. Sm.

About 15 gms. of soil were distributed in ordinary culture tubes and sterilized at 15 pounds pressure for four hours. Dilution plates poured from these tubes remained sterile. In a similar manner, tubes of unsterilized soil were prepared. These sterilized and unsterilized soil samples were then infested by adding a sufficient amount of a 72-hour neutral beef-broth culture of the pathogenes to moisten the soils.

These cultures, covered with a piece of oiled paper to keep out water, were exposed in the following manner: the tubes of *Ps. tumefaciens, Ps. marginatum, Ps. phaseoli, Ps. beticolum, Bacillus carotovorus,* and *B. atrosepticus* were buried from 10 to 12 inches in the soil in the open at different times beginning October 25, 1927; while *Ps. tabacum* and *Ps. citriputealis* were placed on the surface of the soil in the open on January 12, 1928.

The temperature records between October 25, 1927, and March 10, 1928, were obtained from the official records of the U. S. Weather Bureau substation located at Ames, Iowa. The temperature during October was above freezing. The maximum temperature in November ranged from −5° to 20.5° C., the minimum for the same period being from −13° to 5.5° C. The lowest temperature occurred in December when it dropped to −32° C. on December 7. The minimum temperature for December ranged from −32° to 1.5° C., while the maximum ranged from −1.05° to 5° C. The minimum temperature in the month of January, 1928, ranged from −24.5° to 2° C., while the maximum during the same period was from −17° to 16.5° C. The range of minimum temperature in February was from −18° to 2° C., while the maximum was from −9.5° to 15.5° C. The maximum temperature for the first ten days of March ranged from −0.5° to 12° C., while the minimum ranged from −12.5° to 1° C.

Isolations could be made from only the sterilized soils carrying *Ps. tabacum, Ps. citriputealis,* and *Ps. beticolum* because these organisms were very sensitive to crystal violet bile medium. Isolations from the sterilized soil samples were made, and neutral potato-dextrose agar used. For the organisms other than *Ps. tumefaciens* the bile agar was slightly modified. The changes consisted in reduction of sodium taurocholate from 3 to 2 gms., and crystal violet from 2 to 1 cc. and an addition of 3 gms. of malt

extract. Only the bile agar was used for infested, unsterilized soil samples. The overwintered soil samples were brought into the greenhouse on March 10, 1928, and the dilution plates were made the next day. The pathogenicity of the cultures was proved by inoculating susceptible hosts with the suspected colonies. The results of these trials are presented in table 2.

TABLE 2.—*The pathogenicity of eight bacterial plant pathogenes taken from sterilized and unsterilized loam kept in the open during the winter of 1927–28, as determined by inoculations of susceptible hosts with suspected plate colonies*

Organisms	Condition of soil used for infestation	Date placed outdoors	No. days outdoors	Position of cultures	Pathogenicity of suspected plate colonies on susceptible hosts
Ps. tumefaciens	sterile	Oct. 31, 1927	132	12 in. below ground	+[a]
do	unsterile	do	do	do	+
Ps. phaseoli	sterile	do	do	do	+
do	unsterile	do	do	do	+
Ps. marginatum	sterile	Oct. 25, 1927	138	do	+
do	unsterile	do	do	do	+
B. carotovorus	sterile	do	do	do	+
do	unsterile	do	do	do	+
B. atrosepticus	sterile	do	do	do	+
do	unsterile	do	do	do	+
Ps. tabacum	sterile	Jan. 12, 1928	58	On surface	+
Ps. citriputealis	sterile	do	do	do	+
Ps. beticolum	sterile	Nov. 12, 1927	119	12 in. below ground	+

[a] + = Infections on susceptible hosts.

It will be seen from table 2 that *Ps. tumefaciens, Ps. marginatum, Ps. phaseoli, Bacillus carotovorus,* and *B. atrosepticus* lived over winter in sterilized and non-sterilized soils held in the open, while *Ps. tabacum, Ps. citriputealis,* and *Ps. beticolum* overwintered in sterilized soils. Whether the last three organisms can survive the winter in unsterilized soil is still a question. It should be said that the failure to recover the three last-named organisms may have been due to lack of a suitable selective medium.

SUMMARY

Virulent cultures of *Ps. tumefaciens* were isolated from sterilized infested clay, loam, and sand held in the laboratory for 736 days after

infestation. Infection on tomatoes was produced with colonies obtained from unsealed and sealed unsterilized loam and sand cultures 587 and 669 days, respectively, after infestation, and in the case of clay after 539 days.

Pseudomonas tumefaciens, Ps. marginatum, Ps. phaseoli, Bacillus carotovorus, and *B. atrosepticus* lived over winter in sterilized and non-sterilized loam soil held in the open. *Ps. tabacum, Ps. citriputealis,* and *Ps. beticolum* were recovered from overwintered, sterilized loam soil cultures.

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A COMPARATIVE STUDY OF SCLEROTINIA MINOR JAGGER AND SCLEROTINIA INTERMEDIA RAMSEY IN CULTURE

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Smith (4) first reported in 1900 the occurrence of a small sclerotial fungus which was similar in some respects to *Sclerotinia libertiana* Fckl. and which he found destructive on lettuce in Massachusetts. The sclerotia produced by the fungus were decidedly smaller than those of *S. libertiana.* Smith was unable to obtain apothecia from the sclerotia of this form, but, since in one culture large sclerotia appeared among the small ones, he considered that the fungus was a degenerate form of *S. libertiana.*

Later the same fungus was isolated by other workers from lettuce in the vicinity of Boston and New York; at South Lima, in western New York; at Rochester, New York; Philadelphia, Pennsylvania; and Sanford, Florida. Jagger (1) made a study of the strains collected from these localities and concluded that they represented a species distinct from *S. libertiana,* to which he gave the name *Sclerotinia minor* n. sp. In his original description Jagger characterized the species, as far as the sclerotia are concerned, as follows: "Sclerotia black, irregular, 0.5–2 mm. in diameter, often anastomosing to form irregular, flattened bodies which are several millimeters in length." Since the appearance of the paper by Jagger on this species, the fungus has been found in many localities, including at least one station in Japan, and on several other suscepts besides lettuce.

Ramsey (2), in 1924, described a new species, *Sclerotinia intermedia,* which he isolated from decaying roots of salsify in 1920 and in the following year from decaying carrots. He distinguishes it from *S. libertiana* and *S. minor* chiefly by the intermediate size of its sclerotia at room temperature on standard potato, oatmeal, and bean agar; by differences in measurements of ascospores and asci; and on the basis of its lower temperature requirements for mycelial growth.

OBJECTIVES OF THIS INVESTIGATION

In Professor Whetzel's collection of cultures of species of *Sclerotinia* at Cornell University are various strains[2] which, in the light of present knowledge, he regards as *S. minor* Jagger. They have been isolated from various suscepts from widely separated localities. The writer has undertaken a study of these to determine, if possible, whether the variations in these

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[2] The term strain is here used in the sense of a culture obtained from a given collection of the fungus.

strains indicate merely those differences which the individuals of any species may be expected to show; whether they are differences of racial character within the species; or whether they are of such a nature and of such constancy as to justify the separation of certain of the forms into species distinct from *S. minor*.

Mr. B. Baribeau, District Inspector of the Dominion Field Laboratory at St. Anne de la Pocatière, Quebec, Canada, who isolated and sent cultures of two strains of *S. minor* to Professor Whetzel, called attention to the relatively small size of sclerotia of one of these (S451) when grown at a temperature of 10° C. as compared with sclerotia in cultures of the same strain grown at 18–20° C. The writer has undertaken to determine whether this is true for all the strains of *S. minor* listed below.

As Ramsey (3, p. 604) has pointed out the tendency of *S. intermedia* to form larger sclerotia at the lower temperatures, it was decided to include this species for comparative purposes in our temperature studies.

STRAINS STUDIED

In the following list of the several strains of *S. minor*, the first number is that under which the strain is listed in Professor Whetzel's personal catalogue of his stock cultures. The second number is that under which the specimen from which the strain was isolated is entered in the herbarium of the Department of Plant Pathology at Cornell University. The date is that of the year in which the strain was isolated.

S2, *15517*, on greenhouse lettuce, Irondequoit, New York, 1915.

S3, *16204*, on field grown lettuce, South Lima, New York, 1915.

S86, *15515*, on field grown lettuce, Long Island, New York, 1919.

S219, *15520*, on *Chrysanthemum cinerariaefolium*, Japan, 1924.

S334, *15518*, on sunflower, Ontario, Canada, 1925.

S451, *16205*, on potato, Quebec, Canada, 1927.

S452, *16206*, on sunflower, Quebec, Canada, 1925.

Cultures S2 and S3 are two of the original strains used by Jagger (1) in his investigations. The culture of *S. intermedia* Ramsey (S127) is the original strain on the bases of which Ramsey described the species. It is entered in the herbarium of the Department of Plant Pathology at Cornell University under the number *15508*.

Each of the forms named in the foregoing has been grown repeatedly in triplicate on potato dextrose agar made according to a standard formula (Whetzel 5, p. 230) in both petri dishes and 150-cc. or 200-cc. Erlenmeyer flasks, at temperatures[3] of 25°, 21°, 18°, 12°, 9°, and 6° C. The production

[3] Daily records of incubator temperatures, taken while the cultures were growing, showed that while some were constant, others varied somewhat in both directions from the figure given. In the opinion of the writer, however, these variations were not sufficient to influence materially the results obtained.

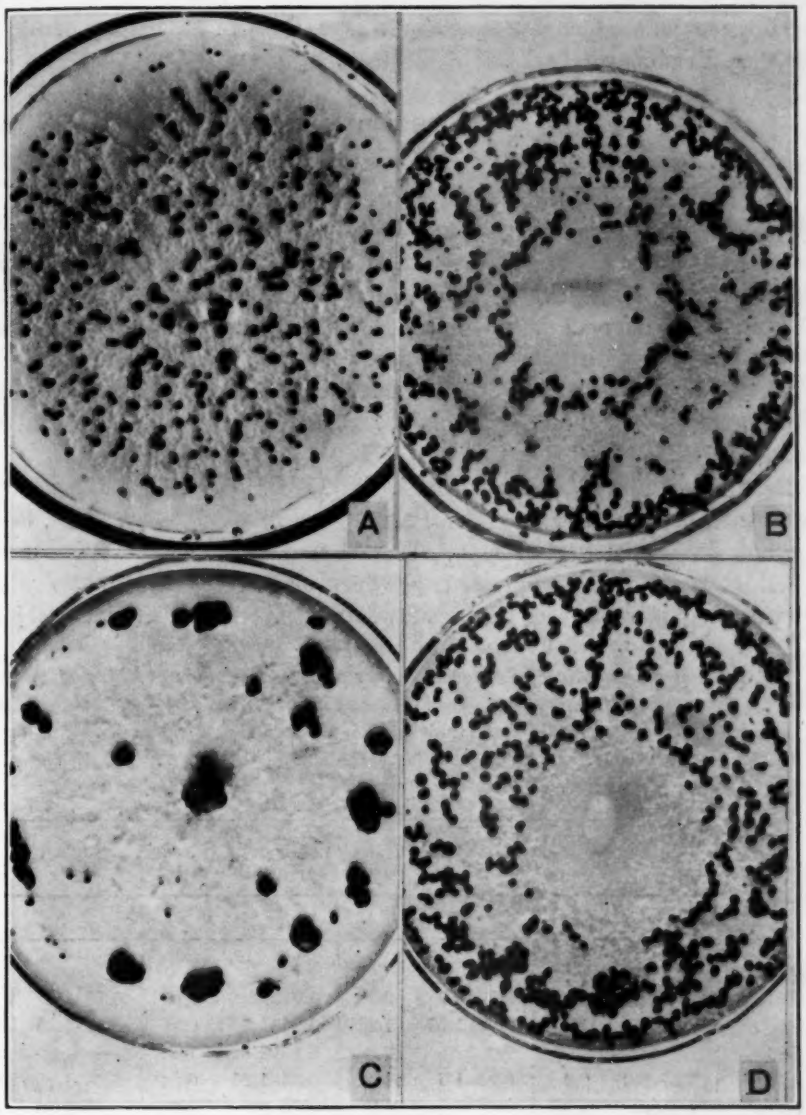

FIG. 1.—A and B. Cultures of *S. minor* (S3) showing the characteristics of race 1 when grown at 25° and 18° C. respectively.

C and D. Cultures of *S. minor* (S334) showing the characteristics of race 2 when grown at 25° and 18° C. respectively.

and characteristics of the sclerotia grown under these conditions have been carefully compared. All cultures photographed for this paper were grown on a layer of potato dextrose agar about 1 cm. thick in the bottom of 150-cc. or 200-cc. Erlenmeyer flasks.

RESULTS

Races of Sclerotinia minor

It was found that the strains of *S. minor* studied can invariably be separated into three groups which are designated as races.

Race I includes S3 and S219. At 25° C. (Fig. 1, A) the sclerotia are relatively large, black, oval or spherical, with little tendency to fuse, 0.25–2.0 mm., av. 1.25 mm. in diameter. The aerial mycelium is appressed, powdery, white, with substratum bare, especially near the margin. At 18° C. (Fig. 1, B) the sclerotia are smaller, black, with tendency to fuse, 0.25–1.5 mm., av. 0.80 mm. in diameter. The aerial mycelium is similar to that at 25° C.

Race II includes S2, S86, and S334. At 25° C. (Fig. 1, C) the sclerotia are formed in fused masses. These masses are relatively few, large, black, irregular in shape, 1.0–5.0 mm., av. 2.44 mm. in diameter. The aerial mycelium is abundant, appressed, powdery or felty, conspicuously white, covering the entire surface of the substratum. At 18° C. (Fig. 1, D) the

TABLE 1.—*Size of sclerotia of Sclerotinia minor and S. intermedia at different temperatures. In each case the maximum, minimum and average size of 50 sclerotia is indicated. Cultures at the higher temperatures matured much more rapidly than those at the lower. These were held in the refrigerator at 3° C. until the latter were mature. Sclerotia were measured at the end of five weeks*

Strain no.	Range in size and average size (in mm.) of sclerotia produced at different temperatures							
	25° C.		21° C.		18° C.		9° C.	
Sclerotinia minor Jagger.								
S2	1.0–5.0	2.34	0.75–4.0	2.06	1.0–3.0	2.0	0.75–2.5	1.4
S3	0.5–2.0	1.6	0.75–1.75	1.22	0.25–1.5	0.87	0.25–1.25	0.66
S86	1.0–4.0	2.04	1.0–3.5	2.0	0.75–2	1.41	———	——
S219	0.25–2.0	0.9	0.75–1.5	1.32	0.25–1.25	0.72	0.25–1.0	0.57
S334	1.0–5.0	2.95	———	——	0.75–2.0	1.2	0.25–1.75	0.7
S451	1.0–3.5	1.64	0.75–2.0	1.14	0.25–1.75	0.88	0.25–1.0	0.56
S452	1.0–2.5	1.51	1.0–1.75	1.34	0.75–2.0	1.24	0.25–1.25	0.6
Sclerotinia intermedia Ramsey.								
S127	0.75–2.0	1.3	1.0–3.0	1.84	2.0–4.25	2.8	2.0–6.0	3.62

sclerotia are not agglomerated, small, black, oval or spherical, with tendency to fuse, 0.75–3.0 mm., av. 1.54 mm. in diameter. The aerial mycelium is scanty, white, with substratum bare between flocci throughout the culture.

Race III includes S451 and S452. At 25° C. (Fig. 2, E) the sclerotia are relatively large, black, oval or spherical, with tendency to fuse, 1.0–3.5 mm., av. 1.58 mm. in diameter. The aerial mycelium is scanty, white and powdery near the center, disappearing toward the margin, at other times so scanty as to give the substratum a bare appearance throughout the area of growth. At 18° C. (Fig. 2, F) the sclerotia are small, black, oval or spherical, with tendency to fuse, 0.25–2.0 mm., av. 1.06 mm. in diameter. When the sclerotia are mature the aerial mycelium is evident only as minute flocci scattered over the substratum.

Attention should be called to the fairly uniform correlation in size of sclerotia, at different temperatures, of the strains of each of these races (table 1).

Relation of Temperature to Size of Sclerotia

Ramsey (3, p. 604), in describing the effect of temperature on sclerotia of species of *Sclerotinia* with which he worked, wrote as follows: "In general, when the temperature is near the minimum growing point for the fungus, the sclerotia have a tendency to be larger than the normal for room temperature. This is particularly so in the small sclerotial forms."

The strains which have been included within the three races already described have been grown at six different temperatures, ranging from 25° C. on the one hand to 6° C. on the other. *Sclerotinia intermedia* Ramsey was at the same time grown at these temperatures.

In cultures of all strains of *S. minor* studied it has been found that the sclerotia decrease in size with lower temperatures (Fig. 2, G and H and Fig. 3, I–M). This holds true from and including 25° C. to 9° C. (Table 1). At 6° C., although a copious growth of aerial mycelium usually occurs, few or no sclerotia are formed at the end of five weeks. On the other hand, in the case of *Sclerotinia intermedia,* the sclerotia are small (0.75–2.0 mm., av. 1.3 mm.) at 25° C., but steadily increase in size with each lower temperature (Fig. 4, N–Q)—just as Ramsey reports—until at 6° C. the sclerotia are larger than at any other temperature of the series (2.75–6.0 mm., av. 4.2 mm., table 1).

Ramsey's statement above quoted would appear, therefore, to apply to *S. libertiana* and *S. intermedia* only. If by the "small sclerotial forms" which he mentions in the second sentence he infers strains of *S. minor,* he would appear to be in error in holding that they tend to be larger at the lower temperatures. The writer fails to find in Ramsey's paper any experi-

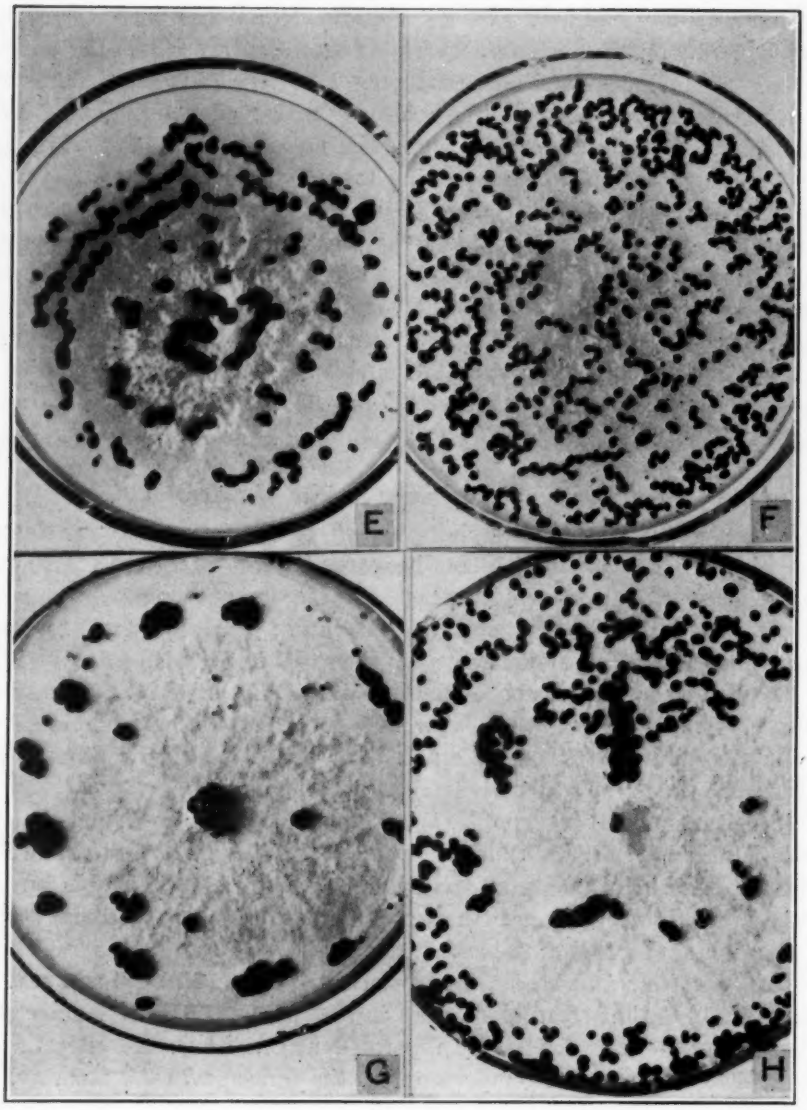

FIG. 2.—E and F. Cultures of *S. minor* (S452) showing the characteristics of race 3 when grown at 25° and 18° C. respectively.

G and H. *S. minor* (S219) grown at 25° and 21° C. respectively, representing the beginning of a series of cultures which illustrates the relation of the size of sclerotia to temperatures in this species.

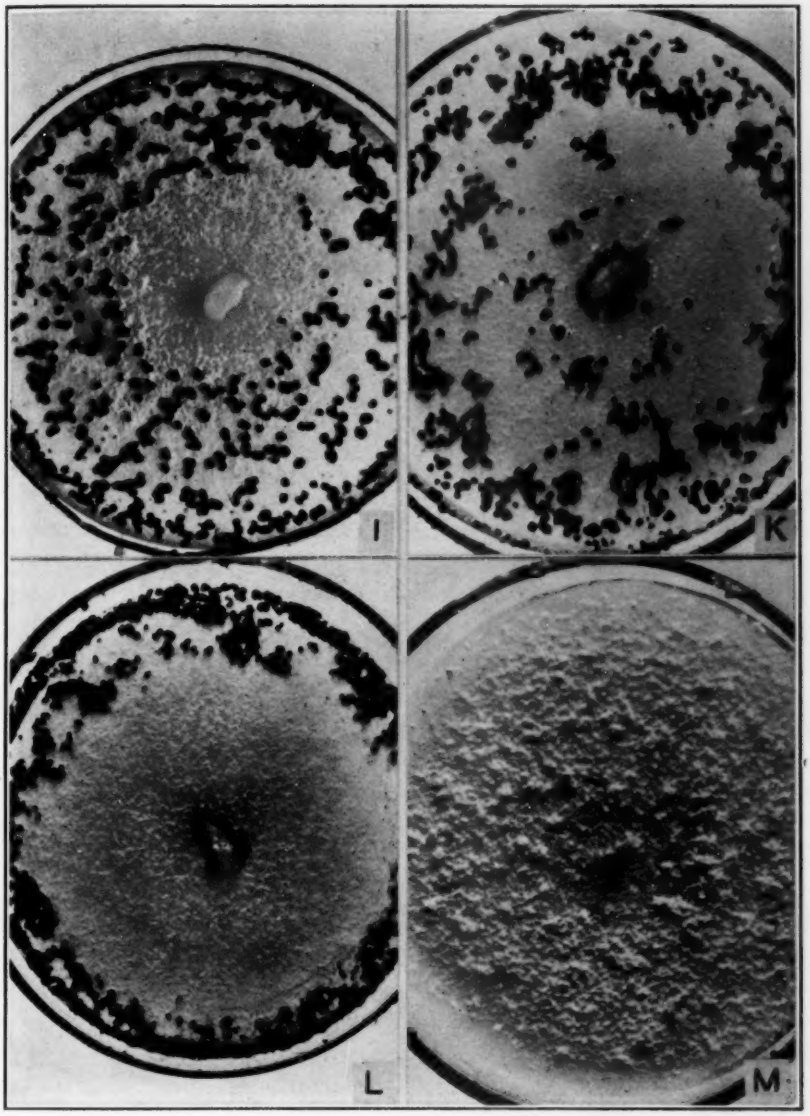

FIG. 3.—I, K, L, M. *S. minor* (S219) grown at 18°, 12°, 9° and 6° C. respectively, completing the series beginning with figures G and H, Plate II.

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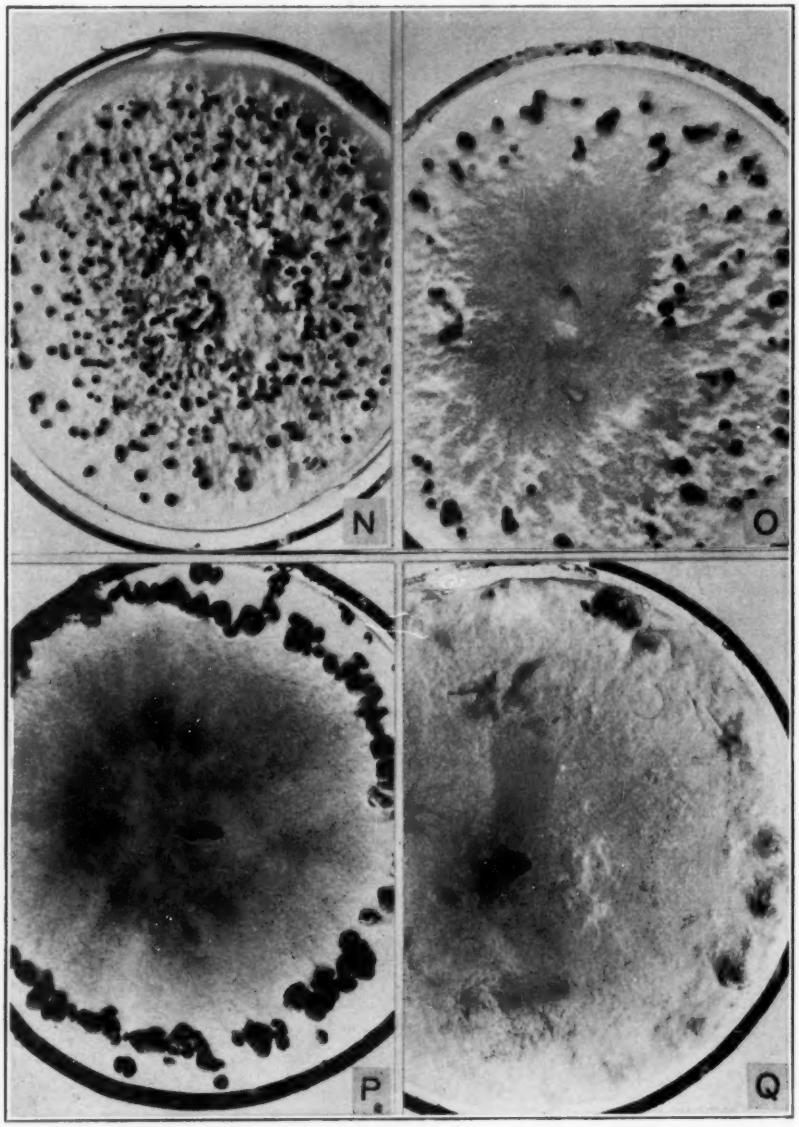

FIG. 4.—N, O, P, Q. *S. intermedia* (S127) grown at 25°, 18°, 12°, 6° C. respectively, representing a series of cultures which illustrates the relation of the size of sclerotia to temperatures in this species.

mental data which warrant such a conclusion. If by "small sclerotial forms" Ramsey refers to *S. intermedia* only, then his data and conclusions agree with what the writer has found for that species.

SUMMARY

It would appear from the results obtained that;

1. Characteristic differences occur among strains of the fungus now designated *Sclerotinia minor* Jagger, which for the present at least may be designated as racial characteristics.

2. The sclerotia of races of *S. minor* vary consistently in size with the temperature at which they develop; the lower the temperature the smaller the sclerotia.

3. The sclerotia of *Sclerotinia intermedia* Ramsey vary in size with the temperature at which they develop, but, in contrast with *S. minor,* the lower the temperature the larger the sclerotia.

These investigations were conducted in the laboratories of the Department of Plant Pathology at Cornell University during the fall term of 1927, while the writer was on sabbatical leave from Darmouth College. The writer is under many obligations to Dr. L. M. Massey, head of the department, for the use of the facilities of the laboratory and many other courtesies. The writer wishes to express his appreciation of Professor Whetzel's generosity in the use of his cultures and for his helpful suggestions during the progress of the work. The writer is also indebted to Mr. W. R. Fisher, photographer of the department, for the preparation of photographs.

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A MOSAIC DISEASE OF THE EASTER LILY

LAWRENCE OGILVIE AND CARL E. F. GUTERMAN[1]

INTRODUCTION

A transmissible virus disease of the Bermuda Easter lily, *Lilium harrisii* (*L. longiflorum* var. *eximium* Baker), and of certain Japanese varieties of *L. longiflorum* has recently been described by the senior writer (2)[2]. In addition to this disease, known as "yellow flat" and characterized by very marked downward curling of the leaves and dwarfing of the plants, another disease or group of diseases has been under observation in Bermuda and in greenhouses in the United States. Although our studies on this disease are as yet incomplete, it is thought advisable to present this preliminary paper.

The first exports of lily bulbs from Bermuda apparently took place in 1883 but there is good evidence that the Easter lily was grown in Bermuda as early as 1856. It is a native of the Liukiu Island, south of Japan, and probably reached Bermuda via the United States. About the year 1892 serious diseases made their appearance in the Bermuda lily fields but it was not until the end of the century that a marked decline of the industry became evident. From that time to within the last six years the industry has been, as remarked by E. H. Wilson (3), "a ghost of its former greatness." Details regarding the decline and subsequent resuscitation of the industry will be found in the senior author's paper on the yellow flat disease.

In 1897 Albert F. Woods, then assistant chief of the Division of Vegetable Physiology and Pathology, United States Department of Agriculture, presented a preliminary report of investigations (4), in which he described mainly the disease which is the subject of the present paper. He attributed the disease to weakening of the plants by improper selection and improper propagation, further increased by the attacks of mites, certain fungi and bacteria.

In 1901 Woods published further observations on the disease (5). An excellent colored illustration of an Easter lily affected with mosaic disease accompanies the article.

[1] The junior author is carrying out further investigations on this disease in pursuit of a joint project of The Horticultural Society of New York, The Department of Plant Pathology at Cornell University, The Boyce Thompson Institute for Plant Research, The New York Botanical Garden, and the Bermuda lily growers.

[2] Ogilvie L. A transmissible virus disease of the Easter Lily. Ann. Appl. Biol. **15**: 540–562. pl. 30–32. 1928.

Some further observations on the disease were made in 1915 when bulbs were forwarded to the United States Department of Agriculture by Mr. E. J. Wortley, then Director of Agriculture in Bermuda. Photographs of, and notes on, the appearance of the plants were prepared by C. W. Carpenter. These are now in the files of the Bermuda Department of Agriculture.

SYMPTOMS

The symptomatological picture presented by mosaic-affected lilies may take one of three following forms. These may or may not represent distinct diseases.

Form A. The form shown in Plate I, A, resembles closely the yellow flat disease. The lower leaves are in most cases normal. Above the third or fourth tier the leaves are curled downwards. In some cases they make a complete circle, the tips pointing upwards. The leaves have a shortened appearance due to the curling but are not actually shorter than normal. The color of the leaves is slightly chlorotic in longitudinal streaks usually about 5 mm. in length. As the leaves grow older these chlorotic markings give place to rusty necrotic areas, which ultimately coalesce. Withering of the diseased leaves takes place, as in the case of yellow flat, about two months before the normal dying of the plants. The apparently healthy leaves at the base remain green, while the others die from the base upwards. The characteristic streaking sometimes occurs on one side of the plant only.

The number of flowers is reduced and the flowers themselves are markedly distorted and sometimes show the chlorotic stripes and mottling characteristic of diseased leaves. The perianth segments are puckered and tend to adhere at the tips (Plate I, B). The stamens either do not develop anthers or are twisted and deformed. The pistil is much twisted.

Diseased secondary shoots are apparently always produced by such plants.

Form B. In the mild form pictured in Plate I, C, and illustrated by Woods (5), minute, pale green or yellowish, linear spots occur on the leaves. These dry out, giving rise to necrotic areas. There is not the pronounced chlorotic appearance or marked curling of the severe form *A*, but twisting of the leaves in various directions is common. In this form the flowers are not markedly distorted.

Form C. This form, illustrated in Plate I, D, is less common than the other two. It is characteristic, apparently, of the disease on certain strains of *L. harrisii*. The leaves have long tapering points and show, especially in the case of the young leaves, distinct mosaic-like mottling or chlorosis. In severe cases the leaves are markedly stiff, crack when twisted, and often

split or coalesce in pairs. The flowers are nearly always aborted, the flower parts remaining green and splitting (Plate I, E).

A somewhat similar splitting of the flowers, unaccompanied by the characteristic leaf symptoms, is produced by too rapid forcing or sudden changes of temperature in the greenhouse.

OCCURRENCE UNDER GREENHOUSE CONDITIONS

All three forms of mosaic have been observed by the writers on plants growing under greenhouse conditions. They are figured in the photographs mentioned above, which were taken in 1915 by C. W. Carpenter.

SEASONAL APPEARANCE

The first few leaves to appear above ground are apparently normal, as in the case of the yellow flat disease. Subsequently, the symptoms appear on the young leaves. The disease seems to be sensitive to slight changes in temperature. A mean shade temperature of 70° F. or higher tends to mask the symptoms to a certain extent. Twisting is not pronounced and the chlorotic areas become much less marked. If cold weather supervenes, the twisting and chlorosis then occur on all new growth.

From flowering time onward, the symptoms in the field are greatly masked by the increase in thickness and color of the leaves. In severely affected plants, dying of the leaves from the base upwards takes place shortly after flowering time, as in the case of yellow flat.

The disease is most noticeable in shaded situations, *e.g.*, at the edges of fields or among grass. The above statements apply to all three forms of mosaic here under consideration.

SPREAD OF THE DISEASE

Evidence of the spread of the disease is clearly seen in the Bermuda lily fields from the month of January onward. The symptoms appear on the topmost leaves of plants whose lower leaves are apparently quite healthy. Most of the spread takes place during the cooler months of the year when the prevailing temperature is about 60°–65° F. Cases have been observed in which the disease spreads in the greenhouse as well.

The following insects occur most commonly in the lily fields of Bermuda: *Aphis gossypii* Glover, *Aphis ogilviei* Theobald, *Empoa fabae* Harr. (= *Empoasca mali* le Baron), and *Cicadula sexnotata* Fall. No positive results have yet been obtained in the attempted transfer of the disease to healthy plants by means of these insects, by means of the bulb mite (*Rhizoglyphus hyacinthi* Banks), by inoculation with extracted juice, or by rubbing healthy plants with mosaic leaves.

Spread of the disease in the fields is somewhat irregular and does not occur in circular areas, as in the case of yellow flat.

EFFECT ON THE BULBS

The disease causes a slight reduction in size and a flattening of the bulb similar to that caused by yellow flat. The mild form (*B*) has little effect on the bulb. For this reason bulbs affected with this form of the disease are not detected in the packing houses and are therefore shipped.

CONTROL IN THE FIELDS

Successful control of the disease has been achieved in the Bermuda lily fields by careful roguing as soon as symptoms make their appearance on the plants. Many of the stocks there are now remarkably free from mosaic infection.

It is a common custom of the better growers to mark their best and most healthy plants for "shelling down," the shells from these yielding healthy stock.

An inspection of the lily fields is now carried on by the Bermuda Department of Agriculture from March onward. This year (1928) not more than 5 per cent of mosaic plants were allowed in the fields. Growers whose stock showed higher than 5 per cent of mosaic plants were not allowed to export their bulbs.

DISCUSSION

It is probable that the disease here reported should be classified as a virus disease of the mosaic type. This is suggested by (a) the presence of mosaic-like chlorotic areas, (b) the susceptibility of diseased plants to changes in temperature, high temperatures tending to mask the symptoms somewhat, (c) its occurrence in certain strains of lilies, (d) the apparent non-recovery of affected plants, (e) the efficiency of roguing in its control, (f) its similarity in several respects to yellow flat, a transmissible virus disease, and (g) its similarity to a mosaic disease of *Lilium auratum* Lind., which has been shown to be transmissible (1).

SUMMARY

A virus disease of the mosaic type is reported on the Bermuda Easter lily (*Lilium harrisii*).

The symptom pictures of three distinct forms *A*, *B*, and *C* are described. These may or may not represent distinct diseases.

The disease has been observed under greenhouse conditions as well as in the field. The symptoms are partially masked by temperature higher than 70° F.

Observations in the field and in greenhouses give clear evidence that the disease spreads from mosaic to healthy plants. The spread occurs irregularly rather than in circular areas around points of early infection.

Attempts to transmit the disease by means of the following insects have failed: *Aphis gossypii, Aphis ogilviei, Empoa fabae,* and *Cicadula sexnotata.* Negative results were obtained with the bulb mite *Rhizoglyphus hyacinthi.* Attempts at mechanical transmission have also failed thus far.

Successful control of this disease has been achieved in Bermuda by means of careful roguing and by the use of healthy plants for propagation.

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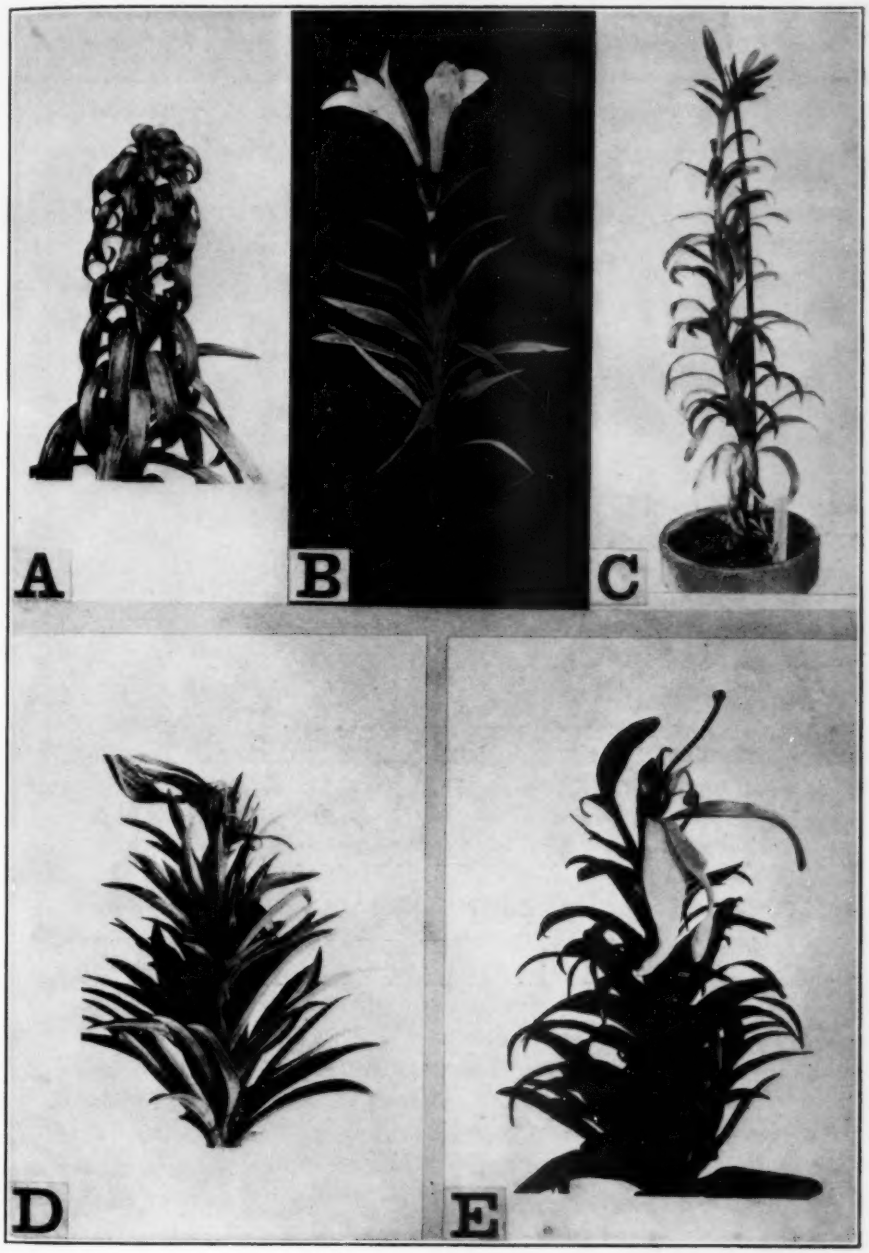
EXPLANATION OF PLATE V

Mosaic plants of *Lilium harrisii.*

A: Plant affected with mosaic *A* or the severe form. B: Typical flower distortion as a result of infection with mosaic *A*. Plant grown in a greenhouse at 75° F. Note absence of marked twisting of leaves. C: A greenhouse-grown plant affected with mosaic *B* or the mild type. D: Plant affected with mosaic *C*. E: Flower distortion and splitting typical of mosaic *C*.


A
B
C
D
E

PHYTOPATHOLOGICAL NOTES

An undescribed leaf condition associated with damping-off diseases of sugar-beet seedlings.—Healthy sugar-beet seedlings have erect straight petioles, while plants injured by damping-off diseases commonly develop bowed petioles (Fig. 1). The bowing of petioles, although beginning with

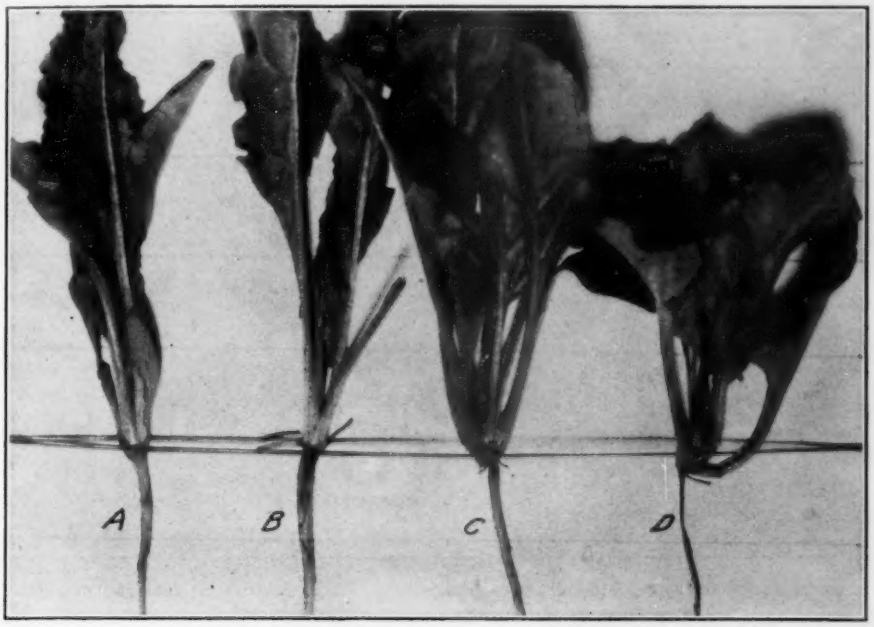


FIG. 1. A and B, healthy sugar-beet seedlings with straight petioles. C and D, diseased sugar-beet seedlings with bowed petioles. The bowing is the result of root infection commonly referred to as black root or damping-off.

the early injury of plants, is most pronounced in the advanced stages of these diseases. The bowing is greatest at the base of the petiole and results in a more open type of top than is common to healthy sugar-beet seedlings.

Damping-off diseases have been known to be the most serious source of injury to sugar-beet seedlings since the beginning of the industry. Such infection is commonly called black root, descriptive of the blackened and discolored roots of diseased seedlings (Fig. 2). The literature dealing with black root of sugar-beet seedlings, based upon observations and investigations in the United States and foreign countries, is voluminous, and no attempt here is made to review it beyond stating that blackened roots, retarded growth, and a pale leaf color have always been noted as the principal symptoms. No reference has been made heretofore to the association of bowed petioles with damping-off diseases.

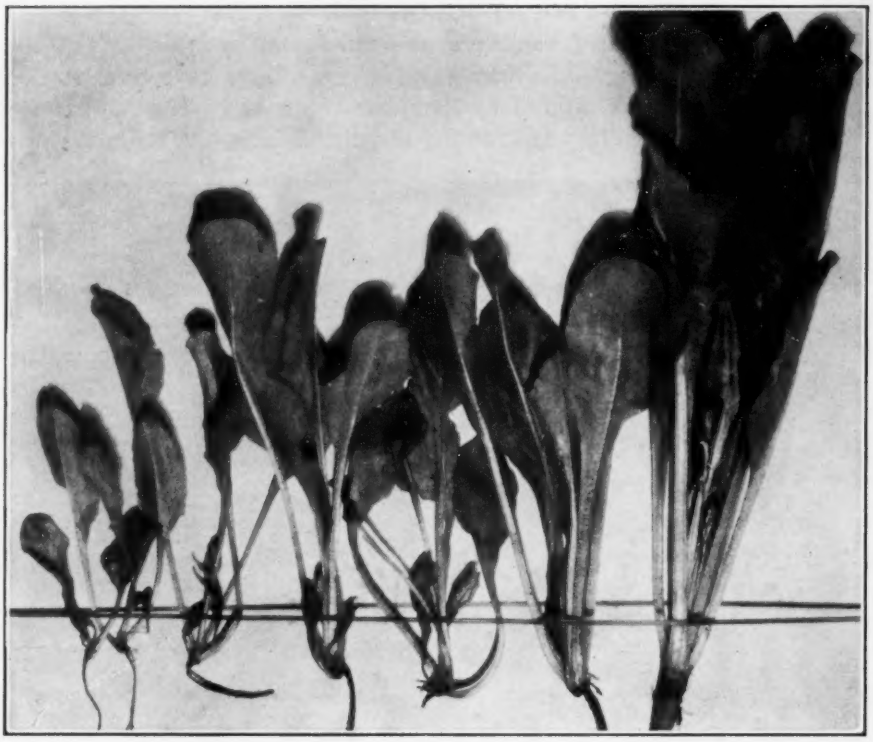


FIG. 2.—Sugar-beet seedlings of the same age. The large seedling at the extreme right is healthy. Note the straight petioles and vigorous growth. The other six seedlings show various degrees of injury by black root or damping-off. Note the conspicuously bowed petioles, blackened roots, and small size of plants.

Sugar-beet seedlings with booked petioles are easily recognized. When the seedlings have from 4 to 6 leaves it is necessary to examine the petioles closely, but when there are from 8 to 10 leaves plants injured by damping-off diseases can be recognized at a distance of several feet.

The writers have selected several thousand beets with bowed petioles from fields in various parts of the western United States and have found black-root injury on each of them.

This method of identification of sugar-beet seedlings injured by damping-off diseases is of practical importance, inasmuch as injured plants can readily be identified without pulling the seedlings from the ground. Sugar-beet thinners can easily remove diseased plants and leave the healthy ones. Very satisfactory results have been obtained by instructing commercial thinners in this means of identification: they soon left very few bowed seedlings. The value of removing diseased seedlings and leaving the healthy ones is very apparent.—S. B. NUCKOLS and C. M. TOMPKINS, Office of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture, Salt Lake City, Utah.